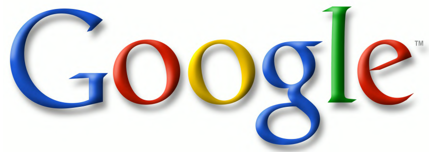


This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

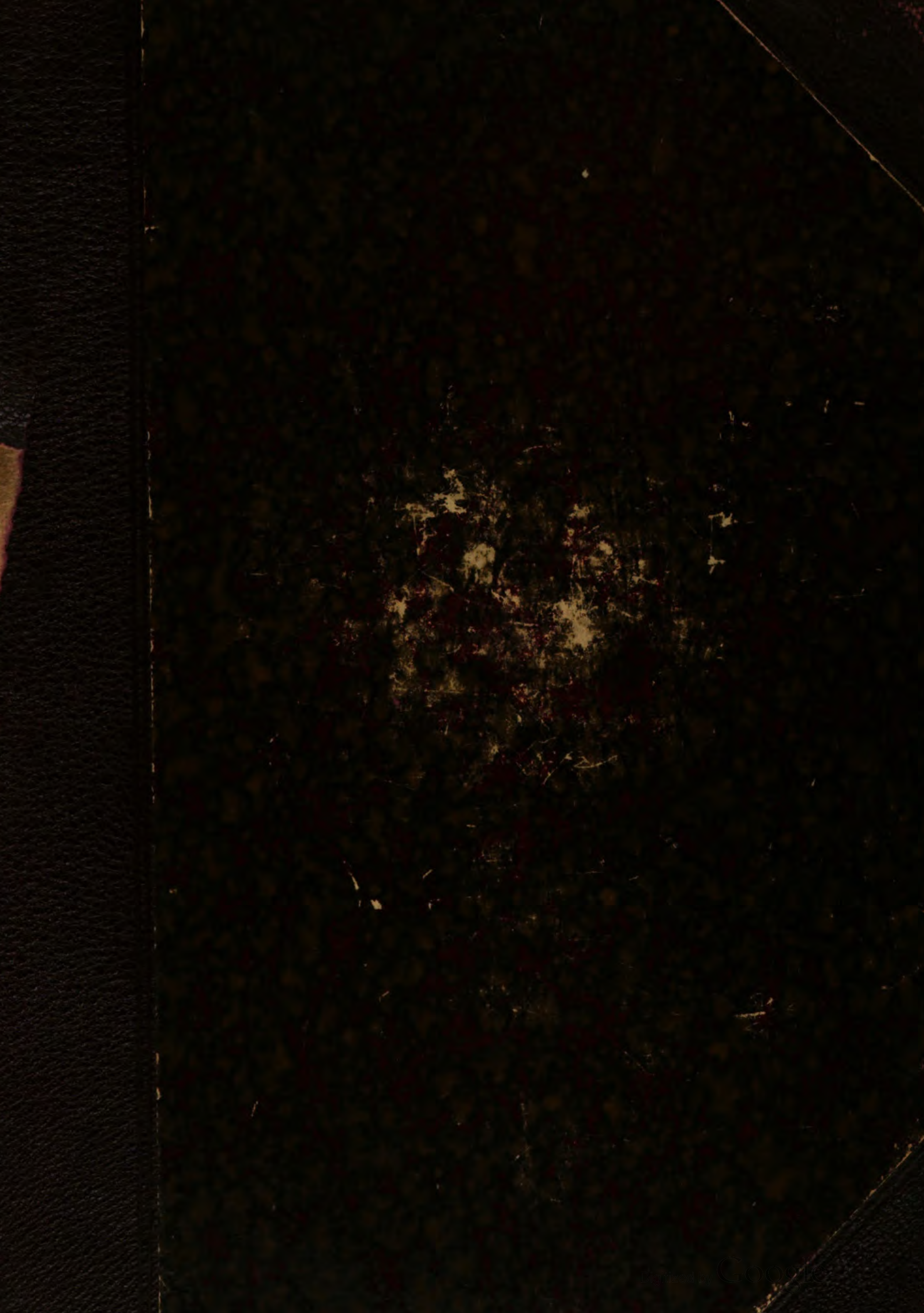
Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.
+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.
+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.
+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at http://books.google.com/



BOSTON

MEDICAL LIBRARY

8 THE FENWAY

Digitized by Google

Digitized by Google

Digitized by Google

Digitized by Google

Digitized by Google

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME LX

BALTIMORE, MD.
1922

Digitized by Google

BOSTON MEDICAL
MAY 18 1922
LIBRARY

MAY 13 1922
M. E. C.

Digitized by Google

CONTENTS

No. 1. March, 1922

An Analysis of the Nervous Control of the Cardio-vascular Changes During Occlusion of the Head Arteries in Cats, *Cora Senner Winkin* 1

The Rôle of the Sodium and the Carbonate Ions and of the Change in the Sodium-Calcium Ratio in the Contraction of the Isolated Duodenal Segment of the Albino Rat. *F. S. Hammett and J. E. Nowrey, Jr* 48

The Rôle of the Change in Hydrogen-ion Concentration in the Motor Activities of the Small Intestine. *Frederick S. Hammett* 52

The Electrical Conductivity of Animal Tissues Under Normal and Pathological Conditions. *George W. Crile, Helen R. Hosmer and Amy F. Rowland* 59

Studies on the Effect of Diet on the Weight of the Hypophysis and Thyroid Gland of the Albino Rat, and on the Action of Their Extracts on the Isolated Small Intestine. *Lyda May Degener* 107

Variations in Output of Bile Salts and Pigments During 24-Hour Periods. Observations on Standard Bile Fistula Dogs. *F. P. Wisner and G. H. Whipple* 119

The Relationship Between Nervous and Hormone Control of the Respiratory Center. *J. J. R. Macleod and S. U. Page* 134

Sensory Stimulation by Saturated Monohydric Alcohols. *Marian Irwin* 151

Studies on the Responses of the Circulation to Low Oxygen Tension. VI. The Cause of the Changes Observed in the Heart During Extreme Anoxemia. *Chas. W. Greene and N. C. Gilbert* 155

Influence of Blood Serum on the Coagulative Activity of Tissue Extracts. *C. A. Mills and Stewart Mathews* 193

No. 2. April, 1922

Studies on the Visceral Sensory Nervous System. XI. The Action of Cocaine and Aconitine on the Pulmonary Vagus in the Frog and in the Turtle. *Nathaniel Kleitman* 203

Studies on the Visceral Sensory Nervous System. XII. The Response of the Isolated Esophagus of the Frog and the Turtle to Certain Drugs. *Z. Bercovitz* 219

The Water Content of the Tissues in Experimental Beriberi. *Dorothy Josephine Krause* 234

Studies in Carbon Monoxide Asphyxia. II. The Growth of Neuroblast in the Presence of Carbon Monoxide. *Howard W. Haggard* 244

Digitized by Google

The Relation of Splenectomy to Growth and Appetite in the Rat. *Arthur H. Smith and Leah Ascham* 250
The Relation of the Adrenals to Fatigue. *F. A. Hartman, R. H. Waite and E. F. Powell* 255
Sensory Stimulation by Unsaturated Alcohols, Polyhydric Alcohols and Chlorhydrins. *Marian Irwin* 270
Successive Stimulation by Alcohols. *Marian Irwin* 274
The Effect of Various Salts on the Outgrowth from Experimental Amoebocyte Tissue Near the Isoelectric Point and with the Addition of Acid or Alkali. *Leo Loeb and Kenneth C. Blanchard* .. 277
Studies on the Physiology of Gastro-enterostomy. I. The Regurgitation of Intestinal Contents in Normal Dogs and Dogs with Posterior Gastro-enterostomy. *G. E. Burget and M. E. Steinberg.* 308
The Pulse in the Portal Vein. *H. Feil and D. D. Forward* 312
Blood Pressure Responses to Hypersystolic Compression of Tissues. *K. A. Martin and H. L. White* 323
The Significance of Hydremia in the Secretion of Urine. *Harry Bakwin* 343
A Study of the Regeneration of the Autonomic Fibers in the Vagus Nerve of the Sheep. *Stanley Ross Burlage* 350
The Secretory Action of the Pancreas in Relation to the Thyroid Gland. I. The Effect of Thyroid Feeding in Rats upon the Secretory Action of the Pancreas. *Hirotoshi Hashimoto* 357
The Secretory Action of the Pancreas in Relation to the Thyroid Gland. II. The Effect of Thyroidectomy in Rats upon the Secretory Action of the Pancreas. *Hirotoshi Hashimoto* 365
A Study of Weight Regulation in the Adult Human Body During Over-nutrition. *Addison Gulick* 371

No. 3. May, 1922

Studies on the Adaptation of Albino Mice to an Artificially Produced Tropical Climate. I. Effect of the Various Factors Composing a Tropical Climate on Growth and Fertility of Mice. *E. S. Sundstroem* 397
Studies on the Adaptation of Albino Mice to an Artificially Produced Tropical Climate. II. Relations of the Body Form and Especially the Surface Area to the Reactions Released by and the Resistance to a Tropical Climate. *E. S. Sundstroem* 416
Studies on the Adaptation of Albino Mice to an Artificially Produced Tropical Climate. III. Effect of the Tropical Climate on Growth and Pigmentation of Hair and the Dependence of these Integumental Functions on the Temperature Coefficient Law. *E. S. Sundstroem* 425
Studies on the Adaptation of Albino Mice to an Artificially Produced Tropical Climate. IV. Effect of Light and Heat on the Resistance of Mice to Acetonitrile. *E. S. Sundstroem* 434
Studies on the Adaptation of Albino Mice to an Artificially Produced Tropical Climate. V. Effect of Humid Heat on the Blood Morphology of Mice. *E. S. Sundstroem* 443

Digitized by Google

Studies in Placental Permeability. II. Localization of Certain Physiological Activities in the Chorionic Ectoderm in the Cat. *R. S. Cunningham* ... 448

Studies in Experimental Traumatic Shock. VI. The Liberation of Epinephrin in Traumatic Shock. *D. Rapport* ... 461

Studies on the Conditions of Activity in Endocrine Glands. IX. Further Evidence of Nervous Control of Thyroid Secretion. *W. B. Cannon and P. E. Smith* ... 476

The Effect of Some Polyhydric Alcohols on the Behavior of Rats in the Circular Maze. *David I. Macht and Giu Ching Ting* ... 496

A Comparison of Waves of Blood Pressure Produced by Slow and Rapid Breathing. *Robert T. Trotter, Philips Edson and Robert Gesell* .. 500

Studies on Alkaligenesis in Tissues. I. Ammonia Production in the Nerve Fiber during Excitation. *Shiro Tashiro* ... 519

Studies on the Conditions of Activity of the Endocrine Glands. X. The Cardio-Accelerator Substance Produced by Hepatic Stimulation. *W. B. Cannon and F. R. Griffith* ... 544

Vasomotor Responses Obtained by Slowly Interrupted Faradic Stimulation of the Thoracic Sympathetic Nerve. *Loyal E. Davis* ... 560

The Isolation of a Substance from Urine Having Properties of Citric Acid: Description of an Apparatus Facilitating the Working with Small Volumes of Gas. *Samuel Amberg and Mary E. Mayer* ... 564

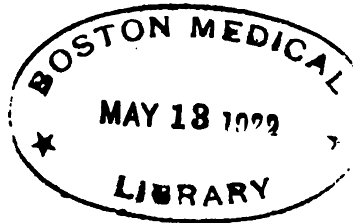
The Influence of Meat upon Physical Efficiency. *S. H. Bassett, Evelyn Holt and F. O. Santos* ... 574

The Gastrin Theory Put to Physiological Test. *A. C. Ivy and J. E. Whitlow* ... 578

Index ... 589

Digitized by Google

Digitized by Google



THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 60 MARCH 1, 1922 No. 1

AN ANALYSIS OF THE NERVOUS CONTROL OF THE CARDIOVASCULAR CHANGES DURING OCCLUSION OF THE HEAD ARTERIES IN CATS

CORA SENNER WINKIN

From the Department of Physiology, Columbia University

Received for publication November 4, 1921

STATEMENT OF THE PROBLEM.[1] The relations dealt with in this study are the cardio-vascular relations found in the mammalian organism under extreme conditions of stress. The procedure of the experiments, occlusion of the head arteries, gives a complete anemia of the brain, and thus produces a profound change in the internal environment of the animal. To this the mammal tends to respond by a series of vigorous reactions. These reactions, moreover, seem to go in a direction opposite to that of the change in internal conditions of a particular group of cells. Thus, with an asphyxial accumulation of carbon dioxide in the medium surrounding the critical medullary cells, there is released an entire series of reactions which, could they all be carried to completion, would reduce the tension of this gas in the body fluids of the cerebral region. Prominent among these reactions is a great and prolonged rise of blood pressure, involving the extreme resources of the organism, tending to send a greater volume of blood to the anemic regions, and hence to decrease the concentration of the carbon dioxide in the nerve cells of the medulla oblongata. In the cat, this anemic rise of blood pressure can be well controlled anatomically, and is suscep-

[1] A preliminary note has been published in Proc. Soc. Exper. Biol. and Med., 1921, xviii, 155.

Digitized by Google

tible of rather exact registration. Moreover, artificial respiration may be maintained throughout the reaction, and thus the activity of the peripheral mechanisms, the heart, blood vessels and internal secretions, be kept free from the central asphyxial changes. Furthermore, under artificial respiration, the reaction may be obtained repeatedly in the same animal. It has therefore offered an opportunity for analyzing the factors involved in such an emergency reaction to inimical conditions in the central mechanism.

Since the work of Ludwig, Cyon and Bezold in the sixties, the importance of the splanchnic vasomotor fibers for the production of extensive changes in blood pressure has been recognized. The related action of the discharge of adrenalin into the blood stream has recently received considerable emphasis. However, the degree to which either the splanchnic constrictor fibers or the secretion of the adrenal glands is involved under such conditions of stress as evoke the anemic rise, has not been evaluated with sufficient accuracy. This study has therefore been concerned particularly with the efferent nervous pathways of the "anemic rise" of pressure: above all, with the degree to which it involves the splanchnic constrictor fibers. The extent to which splanchnic involvement has made for adrenal activity has then been investigated. Finally, the influence of the cardiac innervation, insofar as this may directly effect changes in the level of blood pressure during anemia, has also been examined.

Through the restriction of the effect of the arterial occlusion to the head region alone, the activation of the vascular response by the medulla oblongata is under close experimental control. Accordingly, the central relations of the various nervous levels controlling the efferent channels could also be investigated. Indeed, the analysis of the peripheral factors was in large part undertaken in order to establish more accurately the functional organization of the central nervous mechanism upon which the vascular response depends, that is, the extent to which the peripheral agents executing the vascular responses of the intact animal are activated either by the higher nervous levels, or by the spinal cord alone.

This analysis was undertaken in connection with the studies on the central nervous system carried out under Prof. F. H. Pike, which have dealt particularly with the bearing of its organization on the problem of "spinal shock." In connection with the problems here opened up it was necessary to ascertain the exact nature of the peripheral and central factors controlling the typical vascular response in animals in

Digitized by Google

which either no lesions within the central system were undertaken, or when these were inflicted, no interval for recovery after the operation was allowed. In comparison with such data, a study of the vascular responses after recovery from transection of the spinal cord could be undertaken more intelligently, and the actual changes wrought by the so-called shock effect evaluated with greater precision.

History of the method. Initiated by the very early work on abdominal ligation of Stenson (1) and Swammerdam (2), Magendie and Poiseuille (3) and Sir Astley Cooper (4) in the early nineteenth century worked out the procedure of cerebral ligation, particularly the isolation of the four chief arterial channels to the head, and noted the circulatory changes which followed. Batelli (5) and Hill (6) have given the earlier history of the procedure in some detail.

The experiments of the eighteen fifties and sixties led to the recognition of the nervous organs as the chief agents in activating the changes following arterial ligation: thus the work of Kussmaul and Tenner (7), Brown Sequard (8), (9) and Vulpian (10) on the head area and of Schiffer (11) on the spinal cord. The emphasis of the importance of the medulla for the maintenance of life as given by the work of Le Gallois and its extension by Flourens (12) was still more increased by the localization in the same region of the vasoconstrictor center as soon accomplished by Ludwig (13), Owsjannikow and Dittmar, and soon led up to the most complete studies on occlusion of the head arteries carried out by Sigmund Mayer (14), (15). Mayer not only described the series of changes following anemia with great detail and accuracy, but also recognized that the elicitation of the anemic rise was dependent on conditions of functional conductivity within the brain stem. He also saw that occlusion of the head arteries was comparable to decapitation with the knife, and that the various functions retained following cerebral ligation were all to be attributed to the activity of the spinal cord, notably the residual spinal level of blood pressure of 50 to 60 mm.

Couty (16) produced circulatory obstruction in the head region by the injection of lycopodium spores. This work, contemporaneous with that of Mayer and equally detailed, but carried out under the influence of the earlier work of Goltz (17), (18) and Vulpian, stressed the residual spinal functions, maintained following isolation of the medulla. Subsequent work on cerebral anemia was almost exclusively done from this point of view. Thus the papers of Schlesinger (20), Kowalewsky and Adamük (21), Bochefontaine and Vulpian (22), Mayer (23), attempted to combat this viewpoint by an analysis of the differential

Digitized by Google

effect of compression of the abdominal aorta. Konow and Stenbeck (24) and Landergren (25) more recently stressed the functional survival of the cord in the decapitated animal preparation. The residual spinal blood pressure was analyzed by Pike (26) (1912) who showed that afferent impulses, presumably from skeletal muscles, were responsible for it. His observation that a further fall occurs on paralysis of skeletal muscles by curare has recently been confirmed by Langley, 1919 (27).

A revival of interest in the central relations of the asphyxial picture, particularly to the higher nervous levels, was in part achieved through the reëxamination of the problems of resuscitation of the organism by Stewart, (28), (29), (30), (31), (32), (33) Pike and Guthrie. These observations threw sharply into relief the dependence of resuscitation on the medullary respiratory and vasoconstrictor mechanisms rather than on other organs, which, whatever their importance, were found neither as sensitive nor as susceptible as the medullary and higher cells. The functional activity of the medulla was abolished 15 minutes or more, and in its abeyance, no independent existence of the animal could be reëstablished. An analysis of the conditions of so-called spinal shock was undertaken by Pike (34), (35), (26), who employed the procedure of cerebral anemia, and the vascular response obtainable from it, as a means of comparing the various functional levels of the central nervous system. In this way the central relations, particularly to the bulbar levels, of the vascular response in anemia were clearly indicated. A further extension of this problem is found in the study of Yates (36), in which the response to anemia was used as a criterion of the degree of recovery of the vascular system following spinal transection. These studies bring out the importance of the maintenance of medullary activity as the essential factor in the avoidance of a shock effect and the relative incompetence of the spinal cord in the initiation of significant adaptive responses.

Consideration of the excitatory and depressing effects of the blood gases has led toward a recognition of their importance in influencing the behavior of the medullary cells. The literature of the subject is reviewed by Bethe (37), Hill and Flack (38), Hasselbach (39). Pike, Coombs and Hastings (40), (41) have pointed out the adaptive nature of the nervous changes induced by a rapid lowering of CO_2 tension in dyspneic blood, and have suggested that in thus acting in a direction opposite to environmental change, the organism meets the conditions by adjustment of physical equilibrium as prescribed by le Chatelier's

Digitized by Google

theorem. Mathison (42), (43) has shown the very much greater sensitivity of the medullary over the spinal cells in their response to the asphyxial agents such as increased CO_2 or lactic acid, or decreased oxygen. Pike and Scott (44) have discussed the regulation of H-ion concentration in connection with the regulation of mammalian internal environment.

METHOD. In the present study advantage was taken of the reversibility of the procedure of cerebral anemia. The ability to repeat the initial stimulating effect of the insult on the medullary cells was exploited, rather than its abolition of conductivity within them. The specific problems attacked were dealt with in terms of the intensity and duration of the anemic rise, under given central and peripheral lesions. A seemingly significant series of observations on the changes at the periphery could be obtained by means of the pronounced differences in the character of the curves recorded.

Mayer (14) had called attention to the fact that the magnitude of the vasomotor effect under asphyxia could be approached only by the effect of compression of the thoracic aorta, or injection of strychnine. From Mathison's work (42), (43), especially from his conception that all forms of asphyxia are due to definite increase of the acid content of the blood, cerebral anemia can probably be assumed always to be acting at a maximum. The procedure followed was essentially that indicated by Stewart (28). As here used, the emphasis lay especially on the restriction of the occlusion time to as narrow a limit as possible, in order to insure more rapid recovery. Accordingly, the shortest possible occlusion period was uniformly employed and as a routine procedure the head arteries were released as soon as the spontaneous fall of pressure at the end of the response set in.

The experiments were all carried out on cats. Ether was the anesthetic uniformly employed, and administered by tracheal cannula. The purpose of the study was essentially to determine the degree of involvement of the chief factors concerned, rather than their minute evaluation. This has been left for subsequent study. The extensive series of Stewart served as a basis of comparison and control.

The head arteries were all secured outside the thoracic wall, the branches of the left subclavian, separately secured in the axilla, the right carotid, and right subclavian from within the carotid sheath in the neck; the left carotid held the blood pressure cannula. All the arteries were kept under ligatures ready to be occluded by clamps at the convenience of the experimenter. Since there was no interference

Digitized by Google

with extra-pulmonic pressure through the operative procedure, artificial respiration could be dispensed with as long as the medullary cells remained functional.

Prior to occlusion, ether was reduced until various obvious tests of the activity of the brain stem could be secured, the return of a vigorous corneal reflex always being awaited before the circulatory arrest was made. With the elicitation of the corneal reflex, artificial respiration was begun, and the clamps on the arteries immediately adjusted. Care is needed to include all the arterial branches isolated in the clamps.

With the adjustment of the clamps, the entire series of peripheral effects follows; the eye reflexes are immediately lost, and within about 20 seconds the more marked peripheral effects are released. Deep and labored breathing sets in, skeletal convulsions appear, and a sharp rise of blood pressure is recorded which often reaches 200 mm. Hg. or more (fig. 5a). This frequently outlasts the other functions; the pressure may not begin to fall until some 10 to 80 seconds after respiratory failure.

The time from the shutting off of the arteries to the circulatory failure is then taken as the complete occlusion time. On the average, this occupied 3 minutes.

Immediately following reëstablishment of the circulation there is a profound depression of all functions. Blood pressure continues falling markedly when the arteries are released, and finally reaches a level of about 50 mm. No other medullary responses are elicitable at this time. Artificial respiration is, of course, maintained throughout the period of depression, and until such time as the bulbar functions again become evident.

If no further lesions are inflicted, occlusions of 3 to 4 minutes are usually followed by a beginning of recovery within 5 to 7 minutes after release of the arteries. Blood pressure usually starts rising first, and after a rise of 10 to 15 mm. spontaneous respiratory gasps reappear. Pressure continues to rise, respiratory movements become more and more frequent; soon normal pressure is regained and the animal breathes quietly and regularly. Ten to 15 minutes after release of the arteries, pressure is usually normal, vibrissae are erect, and the corneal reflex is again elicitable. At this point, a renewed occlusion of the head arteries may be done and the entire cycle repeated.

The modification of anatomical conditions was usually carried out in the interval of depression following a control occlusion. In this way further etherization was avoided. Except under certain specified

Digitized by Google

conditions, the various lesions did not materially change or delay the picture of the recovery outlined.

THE EXPERIMENTAL RESULTS. *1. The rôle of the splanchnic constrictor fibers in the rise of pressure during cerebral anemia:* Following the work of Claude Bernard in 1848 who showed that the section of the cervical cord caused a considerable fall of blood pressure, Bezold, Ludwig and Cyons (46), (47), (48), (49), (50) measured the magnitude of these changes and showed their dependence on the integrity of the splanchnic system. There was thus demonstrated the relation of the blood pressure changes to the level which is maintained after the continuity of the cord with the brain has been interrupted.

Mall (51) showed that frequently 27 per cent of the blood in dogs was transferred by the splanchnic system, thus explaining the great increase of volume in the extremities during rises of systemic pressure (52). Edwards (53) calculated that 85 cc. of blood in dogs were translocated under splanchnic stimulation. In spite of its probable involvement in the powerful vasomotor response of the anemic rise, very little direct evidence for its participation has been obtained. Hill's (46) reference to the splanchnic nerves in cerebral anemia is, so far as can be ascertained, largely by way of implication. For asphyxia itself both V. Anrep (54) and Cathcart and Clark (55) have argued for considerable splanchnic participation from the dependence on the central nervous system of the adrenalin release obtained. Finally, some indirect evidence for splanchnic nerve involvement has been obtained by section of the spinal cord in cerebral occlusion. Nawalichin (56) found that the vasomotor changes following obstruction of the cerebral circulation were practically obliterated when the cord had been sectioned in the cervical region. The same observation was made by Stewart (28).

In order to obtain any exact, or possibly even quantitative, evaluation of the actual involvement of the splanchnic system, other factors concerned in the maintenance and change of blood pressure must be isolated. Three factors in the nervous regulation must above all be properly controlled. These are (*a*), the indirect effect of the activity of the skeletal muscles; (*b*), the influence of the cardiac innervation; and (*c*), the non-splanchnic constrictor (or possibly dilator) fibers in the vasomotor system.

a. The influence of the skeletal muscles in the anemic rise. The older authors, Mayer and Couty, used curarized animals, rabbits and dogs, for their experiments on cerebral occlusions, and reported anemic

Digitized by Google

rises as great in magnitude and duration as those recorded by Stewart (28) or those herein obtained. The relative volume of blood held in these animals within the splanchnic system, as compared with that controlled by the somatic innervation is, however, somewhat different from that in cats. Little experimental attention was here given to this problem. In one animal, however, curare was injected and a vigorous anemic response was obtained. The occlusion time was normal (3 minutes); the anemic increment, however, was below the average, being only 80 mm. In another cat, both sciatics and the brachial plexuses on both sides were divided. Pressure did not fall after the lesions. Both stellate ganglia were then removed. The animal gave an anemic increment of 100 mm. Hg.

It seems accordingly that the muscular factor is of no primary significance in either the initiation or the maintenance of the anemic rise. The fact that no great depression of the level of blood pressure results in spite of extensive elimination of muscular innervation is interesting in comparison with subsequent results, and effectively contrasts the influence of skeletal innervation and visceral innervation on blood pressure.

b. The influence of the cardiac innervation on the anemic rise The influence of the cardiac nerves on the anemic rise may be exerted in either of two ways. The change in rate and amplitude of the heart beat may affect the output per minute as emphasized by Tigerstedt, (57) or afferent impulses aroused within the heart may affect reflexly the efferent cardio-vascular innervation as discussed by Hill (59). It is conceivable that in either of these ways, or both, the heart may influence significantly the level of blood pressure.

Frank (57), mathematically, and Erlanger (58) by sphygmomanometric measurements, have attempted to show that the output of the heart remained a constant, or in other words that pulse pressure times pulse rate remains a constant. Wickwire (60) has shown that the usual compensatory changes in heart rate to a change in the systemic blood pressure may be absent in deep anesthesia or in cases of restriction of the volume of blood flow to the brain. Under normal circumstances, Erlanger's statement probably holds true, but may not necessarily apply under critical conditions.

1. Effect of the vagus. Mosso (61), Couty and Stewart found that following the first short rise in blood pressure (which in the intact animal is never very great) there is a considerable slowing of the pulse. As long as this slowing of the pulse persists, pressure ceases to rise, and

Digitized by Google

is indeed often lowered. After about half a minute of this effect, the heart seems to break away from this retardation, and the beat is, if anything, accelerated and pressure immediately rises to the maximum level which is maintained until its final fall. The slowing of the heart rate and the depression of blood pressure gives the anemic rise its typical double crest. Both Couty (16) and Stewart (26) saw this double crest disappear on section of the vagi, leaving a smooth curve, which attains its maximum height somewhat more rapidly, but is not otherwise greatly altered in time or intensity.

Bilateral vagotomy has been done only incidentally to other lesions. The results confirm the earlier findings.

2. Excision of the stellate ganglia. Section of the accelerators as the only lesion was undertaken in five cats, all except one dissection being made in the open thorax under artificial respiration. In all cases the entire stellate ganglion was removed. The mass of nervous tissue was secured by a hemostat and this then cut away from all the connections by which it was held, until the hemostat could be removed without tearing. All the records therefore give a picture of the effects obtained by excision of the entire ganglion including, of course, those additional accelerator fibers recorded by Ranson, Spadolini and Wickwire (60), which reach the stellate ganglion by way of the superior cervical ganglion.

Hunt (62) recorded a loss of pressure on section of the stellate ganglia. Wickwire found a considerable loss (60 mm.) on their section, when this was undertaken without a previous vagotomy. In two cats, 1 and 3, a similar depression was noted. In cat 2, however, the fall was only 20 mm. In cat 7, in which pressure was already very low, no change at all was noted.

Section of the accelerators on both sides seems, like double vagotomy, to have a typical effect on the contour of the curve. It also tends to obliterate the double nature of the curve, which then more closely approaches a single peak. Characteristically, section of the accelerators imparts to the anemic rise a marked plateau effect. After a relatively restricted latent period, pressure rises sharply to its maximum level (fig. 1, occlusion 2), near which it is maintained until just prior to its final fall, when it may again strike the greatest height. The anemic increment of pressure for the five cats examined lay between 120 and 160 mm. Hg. Such a vagus effect as made itself felt, curiously enough, appeared somewhat later than when the accelerators were intact, and the slowing was recorded at the crest of the wave at a very

Digitized by Google

high level of blood pressure. Occasionally a sharp depressor effect may be recorded, which is rapidly compensated for; this effect gives an M-shaped appearance to the curve. On the whole, with the stellate ganglia excised, pressor responses are more promptly executed and longer maintained. In six additional cats, section of the accelerators was complicated by other lesions. In the two cases in which it was preceded by low section of the sympathetic chain, an anemic increment of 80 mm. was obtained in each.

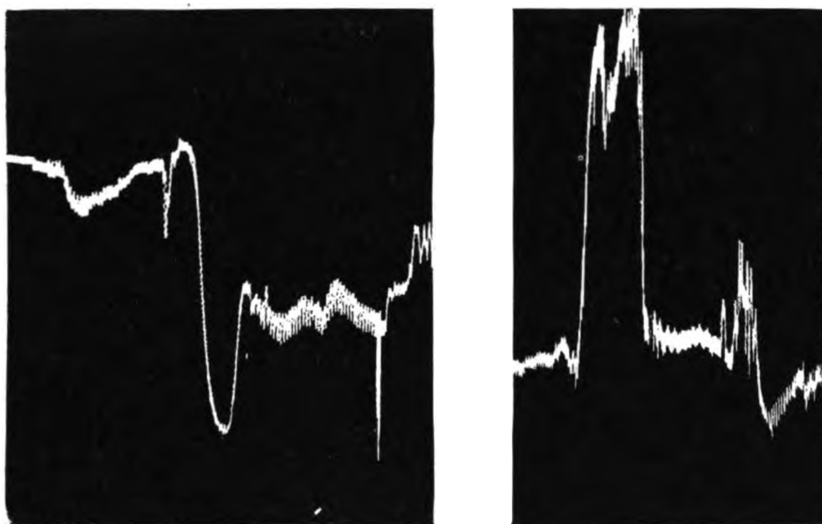


Fig. 1. Cat 4; Occlusion 1. Cerebral anemia, anomalous contour of curve. In this, great fall of pressure replaces the rise ordinarily obtained. The levels of blood pressure before anemia, after anemia and after recovery of bulbar function are shown. Head arteries were released immediately after the rise of pressure, in which the pre-occlusion level was partially recovered. Pressure fell subsequently as low as the lowest point obtained during anemia, but regained 90 mm. above this level with the return of bulbar function.

Occlusion 2: Cerebral anemia; record following excision of both stellate ganglia. When anemia is induced, pressure is 50 mm. lower than after recovery of bulbar function, prior to section of the stellates; M-shaped curve, showing sharp immediate rise of pressure, almost to its maximal height; vagus effect appears at crest of wave. Temporary recovery on release of head arteries, followed by fall to lowest level of pressure (55 mm. Hg. above base line). This low level was three times reached in this animal. Final rise of pressure on renewed return of respiration and other medullary activities.

Each occlusion occupied 3 minutes.

Digitized by Google

3. Excision of the entire cardiac innervation. In three cats the section of both vagi and accelerators was undertaken without any previous lesion. In two of them, section of the vagi was undertaken first, and in both cases a rise of 20 mm. obtained. Subsequent section of the stellates did not appreciably lower (by more than 5 mm.) the original level. The order in which the section of the cardiac nerves is carried out is, therefore, significant for the general level of pressure, and is again in agreement with Miss Wickwire's findings. Several successive curves were obtained from cat 5. The anemic increment was in these cases somewhat reduced, increments of 80 to 100 mm. being obtained after elimination of all the extrinsic cardiac nerves. When all cardiac nerves were sectioned, the curve tended to be smooth, the initial acute rise not being at all delayed. No change in the occlusion time was noted.

Recovery from occlusion after excision of one or both sets of the extrinsic cardiac nerves was uniformly obtained. The time interval of recovery was in no way different from that in normal animals.

In additional cats to be mentioned later, excision of the extrinsic innervation was preceded by a low section in the sympathetic chain. One animal gave an even higher anemic increment (125 mm. Hg.) than is usually obtained after section of the cardiac nerves alone.

In all the curves of reaction to anemia from animals with denervated hearts, pressure was not uniformly maintained at the maximal level. In two cases the pressure dropped immediately; in the rest (4 cases) a plateau was maintained.

4. Effect of the cardiac innervation on the anemic rise. Neither lesion of the cardiac innervation, as a whole, nor of the vagi, nor of the stellate ganglia separately, greatly affects the blood pressure response. Its duration seems to be fairly constant for the given individual tested. Excision of the entire cardiac innervation may reduce the anemic increment in some cases, but the reduction when it occurs does not seem to be considerable.

However, the cardiac nerves seem to have considerable influence on the level of blood pressure in the more detailed relations of the anemic rise, especially in the early part of the reaction. From the results of the section of the accelerators, particularly the abruptness with which an intense rise appears immediately on occlusion of the head arteries, it seems that the conception of the action of the accelerators must be extended. Marey asserted in 1881 that with the vagus intact no very great rise of pressure can be obtained. Indeed, as long as the vagi are

Digitized by Google

functional the maximal anemic increment is not immediately obtained, and cannot be reached in the early part of the occlusion unless the vagi be sectioned. The same seems to follow also for the accelerators since, when they are removed, the vagus cannot prevent the immediate and considerable augmentation of pressure. In the earlier part of the anemic response, the combined action of the entire cardiac innervation seems to effect a considerable check on the rapid rise of blood pressure. This may be due to afferent or efferent impulses, but the accelerators seem to be involved as well as the vagi.

The relations of the cardiac innervation to the second rise of pressure are not so clear. Stewart (28) attributed this in part to accelerator fibers in the stellate ganglion, and possibly in the vagus, but recently Stewart and Rogoff (63) have demonstrated the possibility of producing

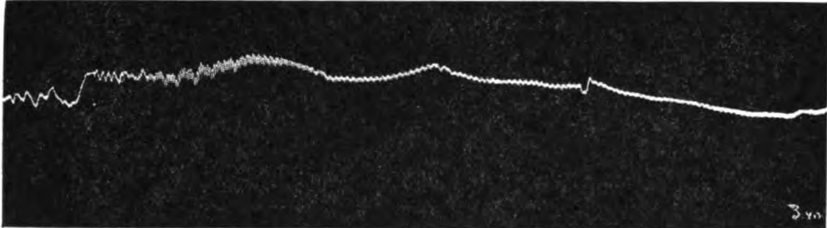


Fig. 2. Cat 23; cerebral anemia. Splanchnic nerves sectioned at their entry to coeliac ganglia. Occlusion time 3 minutes. Skeletal convulsions and respiratory spasms evident. The only factors in the vascular reaction recognizable in the tracing are the changes in heart rate. This is accompanied by a very slight change in level as the heart is breaking away at the usual time from its slow rate. Two respiratory gasps are later imposed on the tracing.

cardiac acceleration by sciatic stimulation even after the heart is completely denervated. In this series of experiments the rise appears very definitely in cats with accelerators removed and vagus intact. It must, therefore, be referable to vasomotor or endocrine effects under these conditions. Ordinarily, there is no break in the curve after double vagotomy, the fall due to vagus slowing being absent. In two cats, however, such a second rise has also been seen when the heart was completely denervated. It seems that the cessation of the vagus effect, while undoubtedly significant, is only one of the factors involved.

In the absence of the influence of the cardiac nerves on the initiation and maintenance of the reaction to cerebral anemia, it seems that we must look to the vasomotor mechanism itself.

Digitized by Google

It is interesting to note, however, that when the animal is no longer intact, and the peripheral resistance has been markedly lowered by a high transection of the cord, these relations are changed. Yates (36) has observed that cats which showed a considerable anemic rise after recovery from such a section, completely lost their ability to react to cerebral anemia following a subsequent excision of the stellate ganglia. However important for all practical purposes the vasomotor control may be, the considerable involvement of cardiac factors in the integrated response, particularly in the event of injury to the vasomotor nerves, must not be overlooked.

c. *Influence of the splanchnic nerves on the anemic rise.* The wide distribution of the splanchnics, gives a possibility for various lesions within the system. Section of the splanchnics was therefore undertaken *1*, in the base of the sympathetic chain before leaving the thorax; *2*, in the abdomen, just prior to their entrance into the coeliac ganglion; *3*, in various levels of the spinal cord in the thoracic region.

The anatomical relations of the splanchnic outflow in the cat have been described by Langley (64), who concludes that the fibers destined to enter the splanchnic nerves leave the cord in large part below the level of the sixth thoracic, though occasionally fibers can be traced at the level of the fifth and even fourth thoracic. Langley's statement appears based only in part on his own observations, and is largely founded on the work of other investigators embodied in the papers quoted. Several authors included higher levels for the effects studied based on experimental rather than anatomical evidence though all have stated that the effect elicitable is relatively slight. Bayliss and Starling gave 3rd thoracic as supplying the portal circulation, Bradford, the 3rd thoracic as supplying the kidney; and Schäfer and Moore, 3rd thoracic as supplying the spleen.

In a more recent study on cats Ranson (65) has re-investigated the problem. He confirms Langley's findings and considers the 4th thoracic the highest limit of the splanchnic outflow. Ranson's material, however, was in part restricted to animals in which only the levels below the 6th thoracic were examined. Ranson has investigated further the level at which the splanchnic nerve leaves the sympathetic chain. In far the greater number of cases (13 out of 17) the nerve was given off between the 1st lumbar and 13th thoracic ganglion, in the remaining four cases, the nerve left between the 1st and 2nd lumbar ganglia. The relation of this branching to the diaphragm was not stated.

Digitized by Google

1. Lesions within the splanchnic outflow: Section of the sympathetic chain; thoracic section of the splanchnics. In 12 animals the splanchnic outflow was interrupted in the lower thorax. Under artificial respiration, a low midventral incision was extended bilaterally on either side of the diaphragm, and the lungs held back while the sympathetic chain was isolated and sectioned.

Section of the sympathetic chain below the level of the 8th or 9th thoracic vertebrae usually gives a very marked fall of pressure. When the splanchnic branch from the sympathetic chain itself is cut, this depression amounts at least to 80 mm. Hg. In spite of this low level of pressure, spontaneous respiration is not usually lost, and when ether is reduced, eye reflexes and other skeletal responses are readily elicitable. The condition of the animal, however, is precarious, and prolonged operative manipulations with too great a depth of anesthesia will readily cause complete loss of the bulbar responses. This precarious condition is in fact met with in all extended lesions within the splanchnic system, and offers some difficulty in the further manipulation of the animals.

Occlusion of the head arteries in this series generally gave a relatively vigorous response. The intensity of the response varied, the degree of variation from the normal being dependent apparently on the nature of the lesion.

Group I. In these animals section of the sympathetic chain was undertaken in its lower levels, post-mortem examination showing no lesion above the level of the 8th thoracic. In two of these animals autopsy showed the lesion incomplete on one side, thus amounting largely to a unilateral injury. The anemic response obtained in four of these animals was very considerable, the values being 100, 120, 140, 150 mm. Hg., respectively. The contour of the curves was typical of the normal anemic responses, and the rise of pressure easily over-reached the original control level of blood pressure. All these cats showed a normal recovery from the occlusion. In nos. 12 and 15, excision of the stellate ganglia was done subsequent to recovery and a third occlusion obtained. Cat 15 that had shown an unusually vigorous response in its first occlusion gave an increment of 125 mm. Hg. after excision of both vagi and both stellates. The thoracic chain was sectioned at the level of the 8th and 9th thoracic on one side, between the 10th and 11th on the other. Cat 11 was slightly different. The original depression of blood pressure after section of the chain was 80 mm. Hg.; the anemic increment was somewhat reduced, amounting only to 70 mm. so that

Digitized by Google

the anemic rise fell short of reaching the original level. The cat recovered, however, and subsequently made up the 10 mm. difference in an anemic rise obtained after the stellate ganglia had been excised. The greater splanchnics may have been involved in this case.

Group II. This comprised the remaining 7 cats of the series. In all these animals a complete bilateral section of the splanchnic nerves was done in the thorax between their branching from the sympathetic chain and before their entry into the diaphragm. On cutting the splanchnic nerves the initial fall of pressure was great, averaging 80 mm. In four cats the effect of occlusion was well marked, the curves differing from the normal only in a slight reduction of the anemic increment of blood pressure, this being 70 mm. in three cases. In these cases also pressure did not reach the level held prior to section.

In the three remaining cats of the series a still greater depression of the anemic response was obtained. Cat 20 gave a most complete picture. The anemic rise reduplicated all the characteristics of the normal response on a smaller scale. A vagus effect appeared prominently. The maximum anemic increment of pressure in these experiments was 40 mm. When pressure fell spontaneously after occlusion it reached the identical level maintained after section of the splanchnics prior to occlusion. Low section of the spinal cord at this time induced a further fall of only 10 mm. Hg. In cat 22 the accierators were also removed, and an even greater depression of the anemic response was obtained, the entire change of level on occlusion amounting to only 5 mm.

Cat 21 was slightly anomalous but yet highly instructive. The animal showed a great resistance to anemia, and it took some 15 minutes before the respiratory and vasomotor responses fully faded out. At first the record clearly approximated that of cat 20, an initial rise of 30 mm. being shown. With the persistence of the bulbar functions, however, there was reproduced on a different scale, the wide oscillations procurable in all animals difficult to asphyxiate. At first the vasomotor oscillations were slight and rather irregular, but they gradually developed into large and rapid waves in which the greatest excursion of blood pressure was developed, amounting to a fluctuation of 60 mm. at the height of the response. The level of blood pressure from which these oscillations developed was not raised, the whole response being simply recorded within this maximum variation of 60 mm. This offers a striking contrast to the analogous records of incomplete occlusion periods of similar length obtained in intact ani-

Digitized by Google

mals. In such animals the level of pressure shows similar oscillations, but these vary within a much greater range, usually approaching 200 mm. difference in level. No recovery of bulbar functions was elicited from any of these animals. That this was not the necessary consequence of a lesion at this level, but merely an indication of the precarious conditions of animals exposed to this double lesion, is shown in the following experiments.

Section of the sympathetic chain; abdominal section of the splanchnics. Although the blood pressure response is seriously reduced by section of the splanchnic nerves above the diaphragm, a slight degree of response still seems elicitable. It seems possible, however, completely to eliminate all rise of blood pressure as the result of bulbar anemia, while maintaining all other evidence of medullary activity, by section of the greater splanchnic nerves in the abdomen.

Dissection for the splanchnics in the abdomen was made by the method indicated in Sherrington's *Mammalian Physiology* (66). The incisions were made from the back, through the latissimus dorsi muscles, and the nerves were cut just before their entry into the coeliac ganglion. The identity of the nerves was first tested by electrical stimulation with shielded electrodes.

A striking example of the result of this section was obtained in cat 23. In this animal (fig. 2) the greatest excursion of blood pressure amounted to 10 mm., yet all other effects of occlusion were noted. An asphyxial effect on the vagi appeared in the pressure curve followed by a very slight improvement in the level. From this point on, however, pressure fell very gradually, until, at the end of 3 minutes, it remained constant. In this very gradual fall, pressure reached a level some 20 mm. below that of the original pressure before occlusion. After digital compression of the abdominal aorta, spontaneous respiration returned in this animal. When respiration had become completely reëstablished and a corneal reflex again obtained, the trachea was clamped. No asphyxial rise of pressure to speak of was obtained, the entire subsequent variation of pressure being well within 20 mm. Hg. Respiratory waves and some vagus effect were recorded; failure of the heart soon followed.

Section of the thoracic spinal cord. Section of the spinal cord was undertaken in 16 cats. The laminectomy was carried out immediately following tracheotomy, the wound temporarily closed by hemostats and the head arteries then prepared for ligation. Finally the cord was sectioned, and blood pressure allowed to reach a constant level

Digitized by Google

before inflicting any further lesions. Several successive sections of the cord were frequently carried out in the same animal before occlusion was produced.

Section of the thoracic cord was carried out at various levels. The effect of section varied considerably with the level of the lesion, and to some extent also with the individual animal. Certain results, however, are patent. Lesion in the lowest levels of the thorax elicited only a slight permanent fall of pressure, and did not seriously affect the anemic response. Lesions in the midthoracic, at the level of the 8th thoracic and 9th thoracic vertebrae were more apt to elicit a profound fall of pressure, and seriously to reduce the anemic increment. Lesions in the upper thorax also elicited a great fall on section and often completely

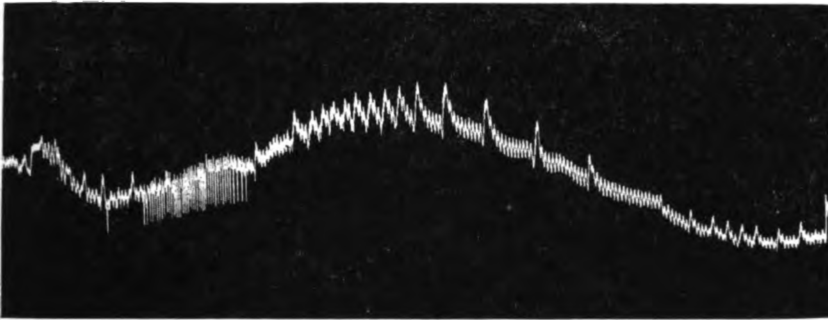


Fig. 3. Cat 30: cerebral anemia. Spinal cord sectioned at the level of the 5th thoractic verterbra. This reaction shows the features of the typical blood vascular response to anemia in every respect, but the level to which the maximal rise of pressure (second rise) attains. The anemic increment here is only 50 mm. Hg. Cardiac effects of slowing and acceleration recorded as usual.

abolished the rise of pressure. There were, however, certain individuals in which even a high thoracic lesion did not evoke a maximum fall of pressure, and in which a relatively vigorous response was obtained even after a high dorsal section. Accordingly the experimental material can be roughly classified into three groups:

Group I. Lesions in the lower thoracic region. Very vigorous responses to cerebral anemia can still be obtained from animals with a lesion at the level of the 10th to 12th thoracic vertebrae. Cat 25 with section at T 10–11 showed an anemic increment of 125 mm. Hg. In cat 24 an anemic response lasting over 5 minutes was obtained, in which the variation of pressure extended over 75 mm. Hg. The contour of

Digitized by Google

TABLE 1

Section of the sympathetic chain and thoracic section of the splanchnic nerves

CAT NUMBER	DATE OF EXPERIMENT	NATURE OF LESION	DECREMENT ON SECTION	ANEMIC INCREMENT	DESCRIPTION OF CURVE
			mm.	mm.	
11	Nov. 20, 1918	Chain sectioned left 9–10 T, right 8–9 T	60	70	Smooth curve, gradual ascent and descent
12	Nov. 19, 1918	Chain in lower thorax right lesion incomplete	No record	100	Normal double peaked curve
13	Nov. 29, 1918 Cat I	Chain in lower thorax left lesion incomplete	70	140	Smooth curve, gradual ascent and descent
14	Nov. 26, 1918	Chain cut above diaphragm	No record	60	Smooth curve, gradual ascent and descent
15	Jan. 28, 1919	Chain sectioned right 8–9 T, left 10–11 T	40	150	Typical curve, great rise long maintained, occlusion time—10 minutes
16	Jan. 14, 1919	Low section of chain and splanchnics	60	120	Typical double curve, pronounced vagus effect, rise long maintained, occlusion time—10 minutes
17	Feb. 1, 1919	Section of splanchnic nerves above diaphragm	80	70	Double curve, marked vagus effect, pressure does not quite reach original level

Digitized by Google

18	Jan. 7, 1919	Section of splanchnic nerves above diaphragm	No record	70	Double curve, marked vagus effect
19	Jan. 16, 1919	Section of splanchnic nerves above diaphragm	80	70	Double curve, marked vagus effect
20	Feb. 8, 1919	Section of chain and splanchnic nerves just above diaphragm	80	40	Marked vagus effect, further section of cord (low) shows loss of only 10 more mm.
21	Jan. 21, 1919	Section of chain and splanchnic nerves just above diaphragm	No record	60	Cat difficult to occlude, respiration fades out after 15 min. Great oscillations of pressure towards end of time
22	Nov. 29, 1919	Section of chain and splanchnics just above diaphragm	80	8	Corneal present just before occlusion, but no appreciable effect of vagus slowing, etc.

Digitized by Google

TABLE 2

Section of the spinal cord at various levels

CAT NUMBER	DATE OF EXPERIMENT	LEVEL OF SECTION	DECREMENT ON SECTION	TOTAL DECREMENT	ANEMIC INCREMENT	DESCRIPTION OF CURVE
			mm.	*mm.*	*mm.*	
34	Apr. 15, 1919	T 10–11	60	30*		No occlusion
		T 8–9	30	60	60	Sharp ascent and descent, pressure just reaches height prior to last section
38	Mar. 18, 1919	T 10	78	44		No occlusion
		T 9–7	20	60	50	Sharp rise marked vagus effect, with loss of 10 mm. pressure
		T 6–2	10	70	5	Some slowing of the heart shown, no increment
31	Apr. 9, 1919	T 2	110	110	40	Double curve, sharp 2nd rise
35	Apr. 16, 1919 Cat II	T 6	55	55 40		No occlusion
		T 4	40	95	20	Depression of pressure only during occlusion; very slight effect
39	Apr. 16, 1919	T 8	68	68		No occlusion
		T 6	30	98	10	No appreciable effect although slight vagus slowing

* 30 mm. again recovered.

Digitized by Google

42	Oct. 22, 1920	T 8	100	100	20	Rather marked slowing
		Vagotomy	30 rise	70	50	Poor record, difficulties with manometer
44	Nov. 19, 1920	T 6	75	75	20	No marked vagus effect, respiratory oscillations show in curve
33	Apr. 21, 1920	T 8	30†	30	30	Marked double rise pronounced vagus effect
		T 6	20	50	10	Effect extremely reduced
24	Feb. 19, 1919	T 10–11	40	40	80	Great fall instead of rise (Cf. text)
		T 8–9	80	120	30	Marked vagus slowing, curve very flat
		Splanchnic nerves cut	No further fall	120	30	Rise obtained on clamped abdominal aorta
25	Feb. 20, 1919	T 11–12	80	80	150	Absolutely typical curve, very sharp rise
29	Mar. 11, 1919	T 11	50	50	70	Marked vagus effect, pressure drops 40 mm. lower after occlusion
30	Mar. 15, 1919	T 11–10	10	Level completely recovered rise above control level		No occlusion
		T 9–5	60	60	50	Very marked curve obtained, vagus effect, second rise accentuated

† Pressure only 80 mm. Hg. to begin with.

Digitized by Google

TABLE 2—*Concluded*

CAT NUMBER	DATE OF EXPERIMENT	LEVEL OF SECTION	DECREMENT ON SECTION	TOTAL DECREMENT	ANEMIC INCREMENT	DESCRIPTION OF CURVE
			mm.	*mm.*	*mm.*	
26	Feb. 22, 1919	T 12	No record		40	Vagus effect appears, curve highly reduced
28	Mar. 8, 1919	L 3	40			Marked vagus effect, pressure
		T 10	No further fall	40	30	Rises only 30 mm. but falls 30 more
		T 8	30	70		No asphyxial increment
40	Apr. 22, 1919	T 9–6	40		40	Very marked vagus effect
		T 6–5	20	60	50	Sharp ascent and descent, marked vagus effect
		T 4	No further fall		42	Sharp ascent and descent, marked vagus effect, pressure drops 30 mm. lower
		T 2	24	84	28	Clear double curve, vagus depression
		Sympathetic chain cut low in thorax	20	104		No asphyxial increment

Digitized by Google

this curve will be discussed below in connection with similar anomalous curves obtained from control records in other animals. In these cats a fall of pressure replaces the usual rise; the variation of level being of the same order of magnitude. In cat 24, despite the great drop of pressure, the original level was regained toward the end of the anemic response at the time usually occupied by the second rise of pressure.

Measurements of loss of pressure after section in the lowest levels of the thorax show a maximal total loss of 50 mm. Hg. Frequently only a few millimeters are lost. In cat 30 the 10 mm. lost after section at the 10th thoracic were completely recovered within 10 minutes, pressure rising even above the level recorded prior to transection.

Group II. Abolition of the anemic response. Lesions of the cord in the region of the 8th thoracic usually entail a rather severe effect; the loss of pressure following this section may amount to 80 mm. Hg. If the fall is as great as this, the anemic response is apt to be seriously diminished. Cat 25, which gave a very vigorous response after section at T 10, showed a further loss of 80 mm. when the cord was sectioned at T 8, the level falling 100 mm. below that held when the animal was intact. The anemic increment after section at T 8 was only 30 mm. Hg. The reduction of the anemic increment to a variation of pressure of only 30 to 40 mm. was seen in five other experiments, (cats 24, 30, 38, 40, and 42) in which section in the region of the 8th to 10th thoracic gave a considerable depression of the level of blood pressure and in which anemia of the bulb failed to evoke an increment of pressure greater than 40 mm. That the vagus is partially responsible for this effect is indicated by cat 42, in which an initial response after section in the 8th thoracic gave an increment of only 20 mm. This increment, however, rose slightly above 40 mm. in a subsequent occlusion after the vagi had been sectioned.

Section of the cord above the 8th thoracic in three animals, cats 35, 39 and 44, gave a very marked fall of pressure in all these cases and no anemic response greater than 30 mm. was obtainable in any one of them. In cat 44, only one section was made at T 6, and no anemic increment at all was obtainable after occlusion. In the other two animals several successive sections were undertaken before occlusion was tested. In cat 39, the first section was carried out at T 7; this was followed by a fall of 55 mm.; 40 mm. more were lost in successive sections ascending to the level of T 4. In cat 35, 80 mm. were lost by section at T 8, and only 20 mm. more by successive sections to T 6. The level of pressure above base line from which only a minimal sub-

Digitized by Google

sequent fall occurs under further manipulations, lies between 35 to 50 mm., the residual pressure maintained by the spinal cord alone.

Group III. Retention of an anemic effect. The midthoracic region is apparently not critical for vasomotor responses in all animals. In cat 34 section at the 8th thoracic gave a loss of only 30 mm. Hg. and an anemic increment of 68 mm. Hg. was obtained. The relatively slight loss of pressure following section at T 7 in cat 39 above mentioned also shows that in some animals the higher levels of the cord are of great importance.

However, the most significant indication of participation of the upper levels of the cord in conveying fibers significant for the vasomotor response was obtained in four additional animals. A strikingly complete anemic rise was obtained from cat 30, in which the cord had been sectioned as high as T 5 (fig. 3). The anemic increment here was 54 mm. In cat 38 a well-maintained response of 44 mm. was obtained on occlusion after section at T 6. In cat 31 a rise of the same magnitude (40 mm.) was obtained after section at T 2. The level maintained after section at T 2 prior to occlusion was 65 mm. Hg. above base line and did not reach the level of 50 mm. until after occlusion. An interesting record of the potency of the higher levels in certain individuals for both the maintenance of blood pressure and its variation is best given in the following protocol—cat 40.

Condensed protocol, cat 40 (pressure here given in level above base line) April 22, 1918

Tracheotomy, blood pressure, cannula, laminectomy, head arteries prepared for ligation.

2:30 Control blood pressure—130 mm. Hg.
2:35 Section of cord at T 8
2:40 Level of blood pressure—118 mm. Hg.
2:45 Section of cord at T 6
2:47 Level of blood pressure—90 mm. Hg., total depression of pressure—40 mm.
2:48 Corneal reflex
Occlusion. Sharp rise of pressure to 114 mm. drop to 90 mm. Hg., great vagus effect, rise again to 130 mm.: *anemic increment—40 mm.*
2:51 Pressure released before spontaneous fall began, gasps immediately return, fall to 80 mm.
2:57 Respiration reëstablished, section of cord at T 5
2:58 Level of blood pressure—70 mm. Hg., total depression of pressure—60 mm.
2:59 Corneal reflex, *occlusion:*—incomplete
3:01 Further manipulation of clamps, immediate rise of pressure to 114 mm., *anemic increment*—44 mm.
3:04 Released before spontaneous fall, pressure drops to 50 mm. Hg., but immediately begins again to recover

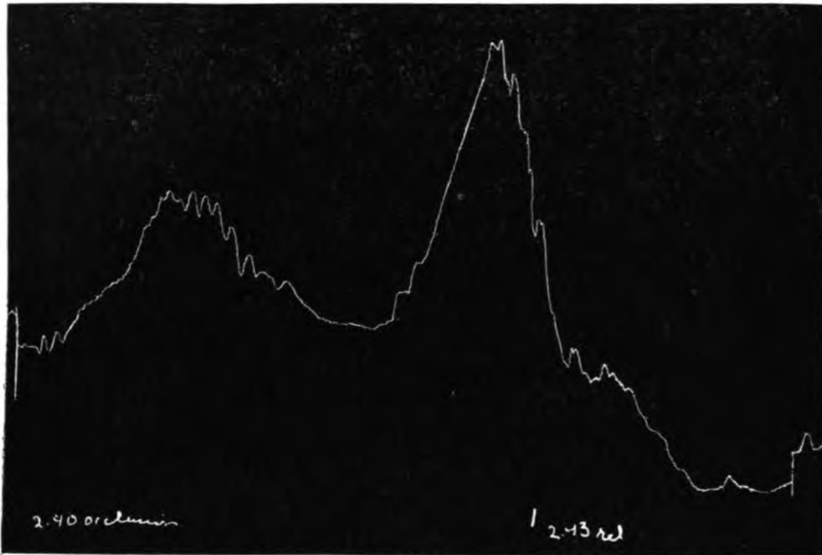
Digitized by Google

3:05 Gasps return, pressure continues rising
3:10 Pressure reaches 90 mm. Hg. again, regular waves in blood pressure curve, respiration reëstablished
3:17 Section of cord at T 4, pressure drops to 70 mm.
Corneal reflex, *Occlusion:* sharp rise of pressure to 100 mm. fall to 60 mm. during vagus slowing, subsequent rise to 112 mm.; *anemic increment*—42 mm.
3:23 Pressure released before spontaneous fall, drops sharply to 44 mm. but immediately begins to rise again
3:25 Gasps return
3:33 Pressure at 84 mm. again, respiration reëstablished
3:39 Section of cord at T 2, pressure falls to 46 mm. Hg. total depression of pressure, 84 mm.
3:40 Corneal reflex, *Occlusion:* initial rise to 64 mm. Hg. fall to 30 mm. during vagus slowing, second rise to 74 mm. Hg. *anemic increment*—28 mm.
3:42 30 Head arteries released before spontaneous fall, pressure immediately falls to 20 mm. but again regains level
3:45 Pressure has reached 54 mm., gasps return, further rise to 60 mm. respiration reëstablished
Thorax opened, artificial respiration administered
4:05 Sympathetic chain cut in midthoracic, pressure fall to 40 mm. total depression of pressure—90 mm.
4:07 Corneal reflex: occlusion, rise to 52 mm. slight fall, rise to 48 mm. *anemic increment*—12 mm.
4:11 Release of head arteries, pressure drops to 30 mm.
4:14 Gasps return, pressure rises to 40 mm. respiration reëstablished
4:55 Splanchnics cut in psoas muscles, no effect on blood pressure, no recovery of level or return of respiration
5:10 Artificial respiration intermitted, pressure drops to base line.

Effect of the splanchnic constrictor fibers on the anemic rise. The burden of the anemic response seems to lie in the vasomotor apparatus, and, if the evidence of these experiments is adequate, almost exclusively on the splanchnic constrictor fibers. Peripheral section of the splanchnic nerves, with its great depression of blood pressure, and the subsequent inability to obtain any anemic increment whatever, speaks strongly, almost unequivocally, for such an interpretation. Additional evidence for the importance of the splanchnic pathway for the vasomotor changes during anemia is the relation of the level of blood pressure after section within the splanchnic outflow to the anemic increment elicitable on occlusion. The data show quite clearly that *the greater the initial depression, the less powerful the response.*

This very definite grading of the blood pressure level to the magnitude of the anemic rise gives a further insight into the anatomical relations of the splanchnic outflow. In the cats examined the greatest

Digitized by Google



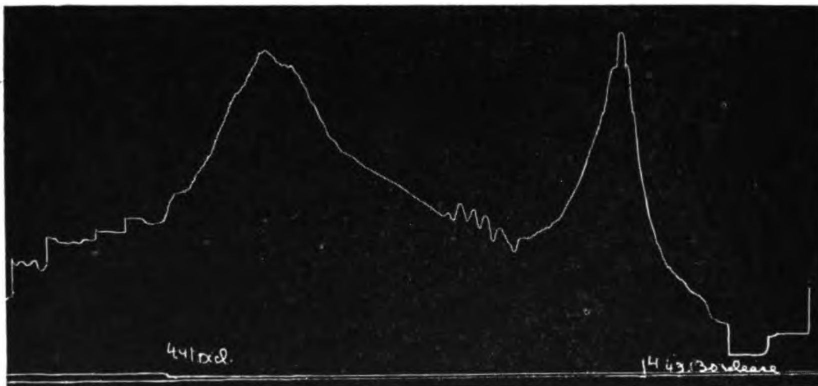


Fig. 4. Cat. 45. Repeated cerebral anemia; double vagotomy; 10th successive occlusion; dissociation of reaction curve into two distinctly separated peaks is shown fully established. Second peak in this occlusion is still considerably greater than the first. Pressure falls very low at the end of the response (30 mm. Hg. above base line). Each curve occupies approximately half the period of the reaction.

Seventeenth successive occlusion: Dissociated curves show little difference in time relations compared with those obtained 2 hours earlier. Difference in contour found in the greater emphasis of the first as compared with the second peak of pressure. The rise in level prior to occlusion represents the recovery of blood pressure after the preceding occlusion following the return of respiratory gasps.

Digitized by Google

average ouflow from the cord to the sympathetic chain is apparently in the region of the 6th to 8th thoracic. Yet the outflow is not restricted to the lower thoracic region, for fibers in the higher thoracic region even as high as the 1st or 2nd, have in these experiments been found of some importance both for the maintenance of the level and the changes of blood pressure. Such a curve as that obtained from cat 30 (fig. 3) shows convincingly that in some animals a good proportion of fibers leave the spinal cord to enter the sympathetic chain above the level of the 5th thoracic vertebra.

While therefore the anatomical findings of Langley and Ranson for the average level of outflow have in the main been verified, the involvement of higher levels already indicated by the various physiological researches quoted by Langley has received a rather striking confirmation.

In such an instance, the physiological evidence may well have precedence over the anatomical, for whereas a small bundle of fibers is most difficult to stain and trace microscopically, a weak physiological effect, when definitely isolated, is quite unmistakable. The involvement of these fibers from the higher levels within the splanchnic system, becomes of particular importance when the entire burden of the splanchnic function is restricted to these levels. Since the very high outflow presumably goes by way of the stellate ganglion, it is necessary to differentiate between the effect of the splanchnic fibers proper and the accelerators. However, in these cats in which an abdominal or high spinal section completely abolished the rise, the cardiac nerves seemed completely impotent against the lowered peripheral resistance.

Accordingly, the vasomotor impulses that travel through the splanchnic nerves to the coeliac ganglion may leave the cord as high as the first or second thoracic. They may, however, stay in the cord throughout the thoracic region and leave it even below the diaphragm. Ranson's results on the very low level at which the splanchnic nerve leaves the chain in cats. is in close agreement with these findings.

There thus appears to be a double pathway for the splanchnic outflow in the thorax, one within the cord, the other outside of it. The outflow of the splanchnic fibers from the cord to the sympathetic chain seems distributed over the entire thoracic region, the relative distribution varying from one individual to another.

In this light the impossibility of abolishing the anemic rise by a section which implicates only part of the splanchnic system is explicable. The wide distribution of the splanchnic fibers would make it difficult

Digitized by Google

to compromise the response by a definitive lesion. Such a lesion could, it seems, be secured only when the section falls sufficiently far out in the periphery or sufficiently high up in the cord, *definitely to interrupt the conduction pathways from medulla to coeliac ganglion.* Unless this interruption is accomplished, the fibers that are left in continuity with the medulla and the periphery are able to initiate an anemic rise which, even if considerably diminished in intensity, repeats all other characteristics of the usual vasomotor response.

To the splanchnic innervation, therefore, the most significant factors in the blood vascular reaction to cerebral anemia can be attributed: the initiation of the rise and the level which this reaches. Since these factors can be controlled by differential lesions within the splanchnic system, the influence of the non-splanchinc vasomotors may be neglected for the purposes of the present survey.

On the influence of the splanchnic system on the maintenance of the normal level of blood pressure: The splanchnic fibers seem involved when the level of pressure is above 50 to 60 mm. for unless a complete interruption of the conduction path from medulla to coeliac ganglion has been demonstrated, pressure returns to a higher level the height depending apparently on the number of fibers in the splanchnic system remaining functional. The level of 50 to 60 mm. is that shown by Mayer, Couty and later workers to be that maintained by the spinal cord alone. Yates also finds this level to be approximately that reached by blood pressure after recovery (2 to 32 days) from high transection of the spinal cord at 8th cervical to 5th thoracic. Her average level of pressure lay somewhat lower than this, between 40 to 50 mm. From Pike's and Langley's studies this residual spinal level appears rather as a skeletal or somatic, than as a vascular or sympathetic phenomenon.

The difference between the residual spinal level and the normal one—a difference of 80 to 100 mm.—would therefore appear as accounted for largely by the action of the sympathetic neurones within the splanchnic system. When the range of variation during anemia is examined, this is seen to be three to four times as great in the animal with splanchnics intact as in the animal which is largely dependent on its skeletal musculature. The variation of pressure in cats with low thoracic section of the sympathetic chain is greatly restricted where the animal was highly resistant to anemia and a period as long as 15 minutes elapsed before the processes activated by the higher levels ceased. Furthermore, as long as the splanchnic system is functional, pressure does not drop below the level of 50 to 60 mm. Hg., however great the variation

Digitized by Google

of pressure and no matter how exigent the inimical conditions. This is well illustrated by the variation of pressure noted in figure 3. The protocol of cat 40 where pressure in maintained in excess of 60 mm. until after a section of the spinal cord at the level of the 4th thoracic, also emphasizes the relation of this level to splanchnic activity.

On some anomalous curves. In a relatively large number of cats (8 in 60) a depression of blood pressure was obtained on occlusion instead of the usual anemic increment. This depression of the level of blood pressure was great, approaching the order of magnitude of the usual positive effect. In six of these cats, pressure fell 100 mm. and more below the original level of blood pressure. Most of these curves represented control occlusions, one example of which has been figured (fig. 1, occlusion 1) in which no previous lesion had been inflicted the vagi being intact in all cases. In one case, cat 24, also mentioned, this depression appeared after low section of the spinal cord. In these cats on occlusion there followed no initial increment, or only a very slight increase in the level (5 mm.). Pressure then continued constant for some 20 seconds. Following this a great and very rapid fall of blood pressure set in from which recovery occurred at about the time ordinarily occupied by the second rise of blood pressure. In this recovery from the low level of blood pressure, however, pressure approached but never completely attained the original level observed before occlusion.

The magnitude of the effect might argue for the involvement of the splanchnic system. As such, it might be aroused by an afferent excitation of the depressor fibers in the vagus. The relation of the depressor to the splanchnic system and also to the discharge of adrenalin (which would of course be involved in all splanchnic excitation) has been discussed by Ludwig and Cyon (48) and Oliver and Schäfer. Bayliss (67) has dealt with the antagonism of asphyxia and depressor stimulation.

On the other hand, the depression of blood pressure, instead of being due to the cardiac innervation set into action through an afferent channel, might be affected directly through a change in the minute volume of the heart, especially under changed conditions within the vagus system. Wickwire (60) has particularly noticed that different degrees of the depth of anesthesia gravely influence the changes in the level of blood pressure due to the vagus system.

II. RELATION OF THE ADRENAL GLANDS TO THE RISE OF PRESSURE DURING CEREBRAL ANEMIA. The extensive involvement of the splanch-

Digitized by Google

nic system in the anemic response makes the activity, or some product of the activity, of the adrenal glands of considerable significance for the problem of its control. Following the discovery by Oliver and Schäfer (68) of the pressor action of injected extract of adrenal tissue, workers have tended to emphasize the close physiological relation of the glands and their pressor activity to the splanchnic system. The literature is extensive (69), (70), (71), (72), (73), (74), (75), but it will not be reviewed at this time. Nor will the literature on the liberation of adrenalin (75 to 90) be considered here. The evidence for the participation of adrenalin in the response to asphyxia and other conditions of stress is also extensive (91 to 98), but its consideration will be left for a later paper. The relation of the contour of the typical curve obtained on electrical stimulation of the splanchnic nerves to the adrenals, and also to the cardiac mechanism, has been dealt with by several authors (104), (105), (106), (107). A further analysis of this contour is also postponed.

Effect of repeated occlusion on intact cats. Elliott's assumption (75) that adrenalin is consumed under conditions of stress makes it conceivable that the rapid repetition of so radical a procedure as arterial occlusion could influence the amount of circulating adrenalin. Since the relation of adrenalin to the myo-neural junction has been experimentally demonstrated, Professor Pike has suggested that, physiologically, it may be associated with the process of conduction from sympathetic nerve fiber to smooth muscle, and directly or indirectly with the processes of excitation in smooth muscle. The work of Keith Lucas would suggest such a possibility (108). Accordingly, such an increase of activity of sympathetic nerve and smooth muscle as accompanies cerebral anemia should lead to a more rapid consumption of adrenalin. This conclusion follows from Elliott's hypothesis of the consumption of adrenalin. The procedure of repeated occlusion has accordingly been attempted first in intact animals in order to reach a control condition of maximal exhaustion of circulating adrenalin, and then in animals in which the adrenal glands had been permanently ligated.

The great resistance of the animals to repeated occlusion has been frequently demonstrated in the experimental material already given. Numerous other evidences of the relative indefatigability of vasomotor responses are found in the literature. Notable here are the analogous experimental conditions in the work of Cushing (99), who found that the process of raising the blood pressure by increasing the intracranial tension, and thus also inducing a partial anemia, could be repeated

Digitized by Google

indefinitely. The difficulty of inducing fatigue of the central vasomotor cells under normal conditions has been discussed in various connections by W. T. Porter (100), (101).

The experiments already described in this series on repetition of occlusion have been complicated by the infliction of lesions in the splanchnic system so that the actual ability of the animals to withstand repeated occlusions, and the effect of this procedure on the anemic response, was not clear. Furthermore, not more than six or eight successive occlusions at most were obtained. Accordingly, in five cats the effects of repeated occlusion were tested. Occlusion was done and when the final spontaneous fall of pressure occurred, the clamps were promptly released and recovery awaited. The corneal reflex was used as before as an index of returned bulbar activity. With its elicitation clamps were again adjusted on the head arteries, and this process repeated several times.

If the occlusion period was not too long maintained in any one closure, it was possible to repeat the procedure practically indefinitely. In the three most striking experiments, cats 45, 46 and 48, the experiments had to be halted arbitrarily because of extraneous reasons, the time consumed being too long. Cat 45 yielded 18 successive occlusions (fig. 4); cat 46, 13 successive occlusions; cat 49, 11 successive occlusions. These experiments lasted over 3 hours in addition to the time necessary for the preliminary operative manipulations which always consumed over half an hour. Cat 46 was intact, cat 45 had suffered double vagotomy, and in cat 48, (fig. 5A) both stellate ganglia had been removed. No marked difference in the behavior of these cats under the test could be noted. In fact, the cats showed a remarkable constancy in behavior. The characteristic occlusion time—2 to 4 minutes—in each individual was retained with considerable uniformity throughout each series. Furthermore, the time needed for recovery of the bulbar functions after release of the arteries was almost uniform for each cat examined. The recovery time which, on the whole, may be said to vary directly with the occlusion time, did not in all cases follow this relation. Cat 46, which gave a constant occlusion time of 2 minutes usually showed a recovery of a corneal reflex within 7 minutes subsequently. Cat 45, however, (vagotomized) invariably showed a 20-minute interval between occlusions. In this interval a well-marked recovery of blood pressure was noticeable and, with the return of respiration, blood pressure rose at least from 60 to 80 mm. above the level after occlusion, before a corneal reflex was

Digitized by Google

obtained. The average level between occlusions was relatively high, pressure seldom falling below 60 mm. Hg.

The first four or five occlusions obtained differed in no very striking detail from control occlusions. The main change from the type of these earlier occlusions appeared gradually. This was a slight delay in the appearance of the first rise in pressure and a gradual increase in the magnitude of this first effect. The fall of pressure from this first level also became more pronounced; pressure dropped to increasingly lower levels at this time on successive occlusions. By the time of the seventh or ninth occlusion the emphasis on this first part of the curve became so well marked that the entire response appeared more as two separate curves rather than one, the two summits in the tracing being very symmetrically distributed both in time and space. The fall of pressure following the initial rise was so great in some of the animals as to approach the base line very closely, dropping to a level of only 10 to 20 mm. Hg.

The characteristic new contour of the rise, once established, is retained in all subsequent tracings in the same animal with great uniformity (fig. 5B). The latter part of the series of occlusion records accordingly shows this new type of anemic rise. The marked drop in the double curve is quite different from the dip due to vagus action seen in the ordinary control pressure curve of anemia. It comes much later (fig. 4); it is also decidedly more abrupt and greater. In fact, it seems much more like an actual collapse of blood pressure. It appears uncomplicated by slowing of the heart. The very definite time relations established in these dissociated curves are striking. Indeed, the supplementary rise, once it has become separated from the initial rise by the marked temporary collapse of blood pressure, is recorded at exactly the same point in all later occlusions obtained in a given animal. This time closely approximates half of the occlusion time of the animal in which it appears, namely, at 1 minute in cats of 2-minute occlusions, and so forth. A decrease of the anemic increment was obtained in the course of the repetitions. Rises of 80 to 100 mm. gradually replaced the original increment of 120 to 140 mm.

One further observation on these cats is worth noting. Post-mortem examination showed that the blood of these animals failed to clot readily. It frequently flowed freely from the carotid artery when the cannula was removed. In a prolonged dissection in one animal, the blood flowed freely from every rupture of a large vessel, even as late as one hour after death.

Digitized by Google

Effect of repeated occlusions in cats deprived of adrenal glands. The procedure of repeated occlusion in the same animal was undertaken in a final series (six cats) in which both adrenal glands were ligated. In each of these animals one control occlusion was made, then by means of dissection through the latissimus dorsi (double incision from the back) the adrenals were isolated and secured by ligatures. No significant fall of pressure was obtained immediately on ligation of the adrenals, thus confirming the observation of Hoskins and McClure (102) and Young and Lehman (103). Following this, the procedure was

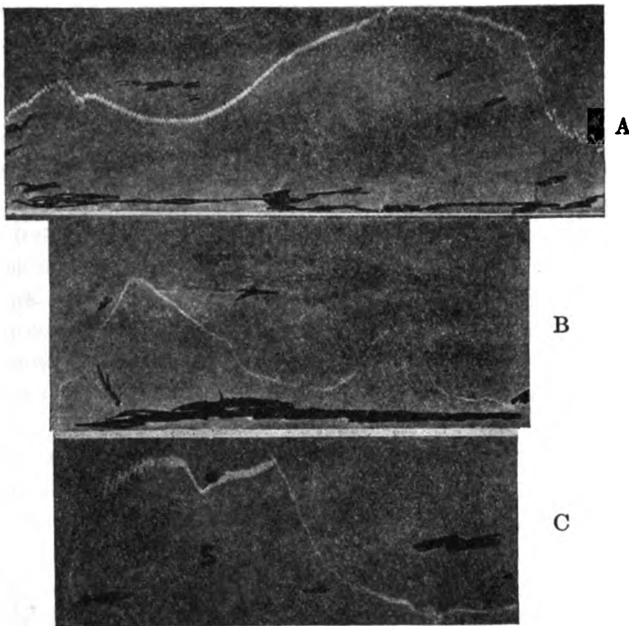


Fig. 5 A: Control curve of 3 minute occlusion. Ligation of the head arteries in the intact and fresh animal. Initial rise is followed by a depression level coming together with a slowing of the heart. High level of pressure maintained throughout the response.

B. Cat 48: Repeated cerebral anemia; stellate ganglia excised; 14th successive occlusion. Dissociation of two peaks very well marked. Level of pressure between occlusions extremely low (20 mm. Hg. above base line).

C. Cat 53: Repeated cerebral anemia; adrenal glands ligated; 7th successive occlusion, obtained just before collapse. Drum revolving at same rate as above. Time of this reaction, 2 minutes. Control reaction in this animal, when fresh and intact had occupied 3½ minutes. Abruptness of initial rise and final fall characteristic of records after ligation of the adrenals.

Digitized by Google

identical to that in intact animals: ether was reduced, a corneal reflex elicited, and successive occlusion of the arteries done as soon as recovery from the last preceding occlusion had occurred. The cats differed somewhat in the rapidity with which the effect of the ligation of the adrenals appeared in the anemic blood pressure curve. In two cats, 52 and 56, the first occlusion following ligation showed little difference, and certainly no curtailment when compared with the control curve. In cat 52 the level of maximal pressure was maintained 2 full minutes longer than in the control. However, in the other four cats, the curves obtained following ligation of the glands immediately presented a marked contrast as compared with the normal occlusion and with the records obtained under repeated occlusion in the control series of intact animals. The characteristic feature of this change appeared at once and was retained until failure of the animal. This was an absolute halving of the occlusion time, and the retention, either of a reduced double curve, or of a single vigorous rise. In the two cats, 52 and 56 already referred to, this same reduction appeared somewhat later in the record. The occlusion time was not halved in cat 52 until the fifth occlusion following ligation of the glands; in cat 56, not until the fourth occlusion. In both these cats there also remained a distinct double rise in the pressure curve, which was not observable in the curves from the other animals. Autopsy showed no difference in the completeness of the ligation in these two animals.

In the cats in which the adrenals had been ligated the complete inability to restore the bulbar functions had to be faced in all cases before nine successive occlusions had been made. There were no exceptions to this early complete collapse in any of the cats observed. Two cats, 54 and 52 already mentioned, gave eight successive occlusions after ligation of the glands. Cat 53 gave seven; (fig. 5 C) cat 57, five; cat 56, otherwise so resistant to a change in its long occlusion periods and retention of normal contour, succumbed after only four occlusions. Only one occlusion was obtained from cat 55. Figured in hours of survival under this procedure, this meant a maximal survival of 2½ hours, a minimal survival of 15 minutes. However, only two cats of the series failed within an hour of the ligation of the glands under successive occlusions. The average survival time was 1½ hours.

Very few indications of the approaching collapse appeared in the record, the only index being perhaps the very low level of blood pressure between any two successive occlusions. This low level was established in all cases immediately after the spontaneous fall of pressure

Digitized by Google

closing the first occlusion that followed ligation of the adrenals. At this time pressure fell to 30 or 40 mm. Hg.—a level 20 to 30 mm. lower than in the intact animals at a corresponding time. In spite of the subsequent return of respiration and other bulbar activities, the pressure remained uniformly low. A return of the corneal reflex was obtained even at this reduced level. The level of blood pressure maintained between successive occlusions varied somewhat. On comparing the amount of recovery of blood pressure in a given animal after occlusion, and the number of occlusions obtainable, it was found that, at least in the extreme cases, a direct variation could be noted. The two very vigorous animals which gave eight reactions after ligation of the glands, cats 52 and 54, showed a recovery of pressure of 40 to 50 mm. during the period following release of the head arteries; whereas cats 55 (one occlusion) and 57 (five occlusions) never regained more than 10 mm. at the time of the return of respiration. Cats 53 (seven occlusions) and 56 (four occlusions) occupied a rather intermediate position, never showing an increase of more than 20 mm. pressure during recovery of bulbar function.

No change in the time needed for the return of medullary activity, as determined by the return of respiration and ocular reflexes, was noted in these animals. As in the control series of repeated occlusion in intact animals, this was not different after ligation of the adrenals from that obtained in the fresh animal. Periods of recovery of from 10 to 20 minutes were recorded, being fairly constant for the given individual.

No significant decrease in the anemic increment was observed, in cats 53 (seven occlusions) and 57 (five occlusions) where increments of 120 to 140 mm. were obtained just prior to failure. These were oddly enough the smooth curves recorded under early collapse. A much more pronounced decrease in the anemic increment was shown in the other animals in which more occlusions were obtained; in cat 52, (eight occlusions) the last occlusion recorded showed an increment of only 65 mm.

The contour of the curves obtained is of considerable interest. Cats 53 and 56 immediately showed a single rise occupying about half the original occlusion time (fig. 6), and this was a smooth unbroken curve. Though the reduction in time was just as manifest in all the other cats, the obliteration of the double nature of the curve was not so clearly marked. In these cats the characteristic contour of the anemic rise as seen in the fresh animal, merged gradually into the smooth curtailed curve following adrenal ligation. The changes most evident were the

Digitized by Google

greater abruptness of the initial rise while the depression of level due to vagus activity was apt to be increasingly delayed and tended to appear on the crest of the wave, somewhat similar to the effect described after section of the accelerators. The precipitous fall which occurs in these animals with ligated adrenals just about half as late as in intact cats then appears as soon as the point of maximal pressure is gained, namely, immediately after cessation of the vagus depression.

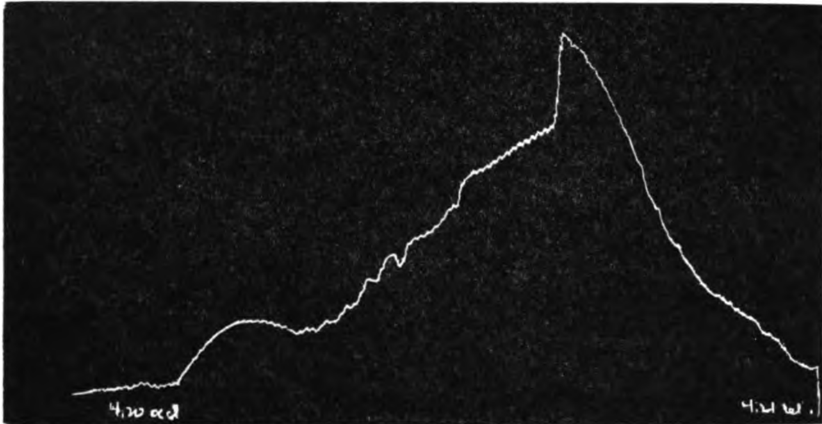


Fig. 6. Cerebral anemia following ligation of the adrenals. Cat. 53. Arterial occlusion immediately following tying off of the glands. Occlusion time, 1½ minutes. Time of control occlusion obtained from this animal, 3 minutes.

Effect of the adrenal glands on the anemic rise. The marked shortening of the anemic response eventually obtained in all the animals in which the adrenal glands had been ligated seems to isolate a further factor concerned in the production of the anemic rise. Apparently the great influence which the splanchnic system is able to exert on the level of blood pressure under the critical conditions of anemia, is due in part to the adjuvant activity of the adrenal glands. These experiments, therefore, bear on the discussion of the emergency relation of the glands, since apparently some involvement of the glands or some product of their activity must be conceded under the extreme condition of cerebral anemia. Furthermore, some clue as to the nature of the activity of the adrenals is given by inspection of the curves obtained.

Loss of pressure. There has been noted a close parallelism between the later curves obtained from all animals suffering repeated occlusion, whether intact or deprived of adrenals. A failure of blood pressure

Digitized by Google

(temporary or permanent) is recorded under both conditions within half the time normally occupied by the blood vascular response. When the animal is intact, this drop of pressure occurs in the seventh or eighth occlusion and in all subsequent curves of a given series. When the adrenals are ligated, it may be established immediately, although this is not necessarily the case. Under these conditions the halving of the response is recorded before four successive occlusions have been inflicted and is found in all occlusions which follow in these animals. This precocious loss of level in blood pressure can therefore be obtained either when the animal has been exposed to rapidly repeated cerebral anemia, or when the activity of the adrenal glands is completely abolished. Accordingly, the main factor in the production of this early failure of pressure seems concerned in all cases with the availability in the blood stream of some product of adrenal activity.

Restoration of level of blood pressure. An examination of the supplementary rise of blood pressure in the repeatedly occluded but intact cats in comparison with the permanent failure of pressure at half the normal occlusion time when the adrenals are ligated, leads to a consideration of the theories of emergency function of the adrenals. This secondary rise is most probably related to the presence of functional adrenals.

The secondary rise was interpreted in a preliminary report of these experiments as an indication of an increased liberation of adrenalin from the glands, and the constant interval prior to its appearance, as a latent period, relatively long, of adrenal secretion. The argument was advanced that these experiments offered evidence confirming Cannon's position on the increased secretion of adrenalin under emergency conditions. However, in view of the presumable consumption of the products of adrenal activity during cerebral anemia already discussed, the conception of the emergency liberation of adrenalin must be somewhat modified. The further discussion of any of the current hypotheses of the liberation of adrenalin must be deferred until further experimental evidence has been accumulated. Two definite statements, however, appear justified by the facts. In the first place, since curves of perfectly normal contour were obtainable in two animals after ligation of the adrenals, an increased liberation or secretion of adrenalin, one or both, is not necessary for the carrying out of the typical blood vascular response to anemia in the fresh animal.

That these results were due to experimental error can hardly be possible since there was seen in these animals a gradual and relatively slow

Digitized by Google

transition of the normal curve into the abbreviated response typical for animals with ligated adrenals. Such a gradual transition is also found in the intact repeatedly occluded animals. In the second place, from the premature failure of the vascular response, after the ligation of the adrenals, particularly in contrast to the secondary rise that is seen in the intact repeatedly occluded animals, the conclusion may be drawn that some product of adrenal activity must be available to make possible the continued action of sympathetic nerve on smooth muscle for any length of time.

Survival after adrenal ligation. In all the work reported on excision of the adrenal glands, sudden death has never been noted. However, when all adrenal tissue is excised, collapse and death follow, the interval of life varying in different animals. The earlier work on cats has been reviewed by Hultgren and Anderson (109), who particularly described the prelethal stage. Elliott (73) recorded the failure of blood pressure in addition to the loss of the pressor reaction in the moribund cat, and in a later paper he has summarized a series of tests given in these conditions, demonstrating a complete collapse of vascular tone. Gautrelet and Thomas, (110) later Hoskins, (111) have confirmed the depression of the sympathetic system on final collapse. Elliott records death with simultaneous extirpation under ether after 14 to 18 hours. Bazett (112) has recently succeeded in shortening this time considerably by decerebration, urethane anesthesia and sensory stimulation. In these animals the fall of blood pressure occurred within a few hours after the operation. Elliott (98), moreover, finds that the animal survives even if the adrenal tissue is separated from the splanchnics. He concludes therefore that, whereas the increase of adrenalin in the blood stream under splanchnic stimulation is not necessary to life, the animal depends for its existence on the continual slow secretion of adrenalin from the medullary cells. Elliott argues that this continual slow secretion is independent of nervous impulses. Stewart and Rogoff (113), (114), however, are unable to demonstrate any appreciable adrenalin output under these conditions.

It seems that the repetition of the extreme procedure of occlusion is able to hasten the onset of complete failure most surprisingly. In the extreme conditions of these experiments Bazett's (97) already curtailed time of survival after ligation of the adrenals is thus further shortened by 6 or 8 hours. The only demonstrable factor in the failure under these conditions is the inability of the sympathetic nervous mechanism to maintain the normal state of the musculature of the blood vessels

Digitized by Google

after complete exhaustion of the reserve of adrenalin in the blood. The necessity for the presence of adrenalin or of some other product of adrenal activity in the blood for the maintenance of vasomotor tone, as asserted by Elliott, seems again confirmed. The failure of blood pressure alone seems able to carry with it the failure of all the other functions.

From the evidence, the relative degree of constriction of the vessel walls seems, to a considerable extent, a function of the amount of some adrenal product in the circulation. The loss of this product seems to mean complete failure; blood pressure stays only a few millimeters above base line when the available supply is low, but an increased liberation, or possibly even a redistribution, may give any degree of tonic contraction of the vascular muscles, reaching to maximum constriction, the entire reaction perhaps depending on conditions at the myo-neural junction.

III. Relation of the splanchnic sympathetic system to the central nervous system. The central relations of the sympathetic system have been tenaciously disputed, and cannot be entered into at length. On the one hand, there has been the view defending its relative independence from the cerebro-spinal axis, originally advanced by Bichat (115), and supported extensively by Volkmann (116). Goltz in his latest work with Ewald (117) subscribed to this view, in his assertion that the sympathetic peripheral ganglia could maintain normal vascular tone, and mediate reflexes quite independently of the central nervous system.

However, the theory that the nervous outflow is essentially dependent for its activity on cells of central, and particularly bulbar origin, has always enrolled some powerful supporters. Two of Goltz's contemporaries, Eckhard (118) and Mayer (119) have defended this conception. Recently Gaskell (120) and Sherrington (121) and still later Ranson, (122), also endorsed it.

Two points in the evidence on cerebral anemia will briefly cover the relation of the medullary cells to the peripheral response. First, the comparison of the splanchnic response with the other peripheral responses, and particularly the skeletal responses as controlled by the medullary or higher cells, under the different functional conditions of the nervous levels in these experiments; second, the behavior of the blood pressure responses under recovery from various spinal lesions.

Comparison of splanchnic response with other peripheral responses. The following table gives the various stages which can be distinctly separated when different functional levels control the animal's reac-

tion. The rough average of the level of blood pressure maintained is given for each period. The exact correspondence of the involvement of the splanchnic system and the degree of functional activity within the medullary centers is indeed striking, especially in view of the potency of the splanchnic system in maintaining blood pressure. It appears from this tabulation that the splanchnic system behaves exactly as do the respiratory, skeletal and ocular responses. When the skeletal responses dependent on the higher levels are in abeyance, the vasomotor responses of the splanchnic system are also absent. At this time, moreover, that is, during the depression between occlusions, the heart rate shows no appreciable change. The level of blood pressure maintained is that shown by Couty (16), Mayer (14), Pike (26) and Langley (27) to be that held as long as the spinal cord itself remains intact. Additional evidence that the depression of functional activity is due to a complete interruption of conduction in the spinal cord, and not to so-called spinal shock, is brought out by the behavior of the animal in passing through these various stages. The bearing of the validity of the shock hypothesis for any conception of the functional organization of the nervous system has been discussed by Pike (123). A shorter statement of this relation is found in Yates's paper (36).

Comparison of somatic and ocular responses with vascular responses

Control of the animal's responses by various nervous levels	*Average level of blood pressure*
1. Normal intact animal: responds as a whole, pupils narrow, corneal reflex, respiration normal...........	120 mm.
2. Head subjected to anemia: struggles—responses under control of stimulated area (head) skeletal convulsions, respiratory spasms, corneal reflex lost.	180–200 mm.
3. Head functionally dead,—animal spinal:—responses under control of spinal cord only no corneal reflex, pupils widely dilated. No spontaneous respiration. No skeletal reflexes elicitable..............	50–70 mm.
4. Recovery of head centers: gradual return of responses controlled by head area, pupils narrowing—no corneal reflex—spontaneous respiration returns after pressure has risen somewhat, but still sporadic. Skeletal reflexes elicitable in part.................	70–90 mm.
5. Recovery completed; animal responds as a whole. Pupils narrow, corneal reflex, respiration reëstablished; functions coördinately, skeletal reflexes present...	120 mm.

When a significant lesion in the splanchnic system has been inflicted by the section of these nerves just before entrance into the coeliac

Digitized by Google

ganglion and the cerebral circulation shut off, no anemic increment is obtainable. However, all other evidences of bulbar activity are present. A vigorous corneal reflex is obtained prior to occlusion, and when the clamps are adjusted, even though the level of pressure may remain more undisturbed than that obtained under many minor manipulations, the other symptoms of the asphyxial response are shown in full vigor. There are marked respiratory spasms, skeletal convulsions, changes in the pupils, etc.

In marked contrast to such a picture are the effects when, from some physiological disturbance, the medulla itself is thrown out of activity. Here the effects of the interruption of functional continuity are opposed to the effect of anatomical separation. Such a condition is present while the animal is still profoundly under the effect of an occlusion that has just been done, or even during recovery from occlusion, when the functions of the brain stem are not yet fully established. If, under such conditions, the animal is subjected to a renewed occlusion, no response at all can be aroused. Generalized asphyxia, inflicted by clamping the trachea and thus acting directly at the periphery is, in this condition, also impotent to produce any effect. Under this general depression there are no skeletal convulsions, no respiratory gasps, and pressure changes are extremely slight, 5 or 10 mm. The heart just quietly runs down. The condition of the eyes remains unchanged throughout.

All the evidence of these experiments therefore would argue not only for a normal release of the rise of blood pressure through the sympathetic outflow, but also for a complete dependence of the activation of the response on the integrity of the brain stem, and the maintenance of the conditions of conductivity within it. The response transmitted by the sympathetic system is functionally exactly on a par with all the other physiological responses. When respiratory movements, eye movements and skeletal reflexes are obtainable, the changes of blood pressure can also be elicited.

The anatomical relations of the splanchnic outflow in its bearing on recovery after section of the spinal cord. The complete dependence on the brain rather than on the spinal cord is well illustrated by experiments on recovery of blood pressure from high section of the spinal cord. Goltz, (17), (18) and later Goltz and Ewald (117) sectioned the cord of dogs in the midthoracic region and found normal blood pressure responses to subsist. These were attributed entirely to the controlling influence of the cord over the sympathetic system. These experiments have been repeated by later investigators, notably Sher-

Digitized by Google

rington (121), (124). In view of the very high level of the cord in which a lesion must fall before it can definitely intercept the connections between the medulla and the splanchnic effectors, there is a possibility that the agency concerned in this recovery is none other than the splanchnic constrictors still in functional continuity with the brain stem.

Four early experiments were carried out with Doctor Pike's coöperation in which a transection in the upper levels of the spinal cord was done aseptically, the animal allowed to recover and then the anemic response tested. In two cats the transection was done at the level of the 2nd thoracic. One animal died within 24 hours before the blood pressure could be tested; the other lived 5 days and was then subjected to the test of occlusion. Blood pressure, however, was very low, the bulbar responses failed immediately and no rise was elicitable. Cat 63, however, in which section at the 3rd thoracic was made, recovered fully and when tested a week later showed a level of blood pressure of 120 mm. and an anemic increment of 50 mm. in the first occlusion. Cat 64 with a section at the 6th thoracic was tested 2 days later. Control level of blood pressure was 80 mm. The anemic increment of the first occlusion was 45 mm.

This problem was subsequently taken up by Miss Yates under Doctor Pike's direction, and has been reported on in detail in an earlier issue of this Journal. Miss Yates (36) found that when one or two segments of the thoracic region only are left intact there is a recovery of blood pressure to an adequate level, and vigorous anemic or asphyxial response is readily elicitable. This recovery is attributable to those medullary cells still in connection with the peripheral splanchnic neurones.

It was on the evidence of Goltz's experiments that Langley applied the name "autonomic" to those peripheral mechanisms supplied by ganglionic connection outside the nervous system which he thought could function independently of the brain. The physiological evidence that is now accumulating would gravely discredit this autonomy, and would tend to place the sympathetic responses in the same category as all others. This is of particular importance in connection with the late appearance of the adrenal effect in cerebral occlusion, even after the reflexes are no longer elicitable. However late its appearance, and however independent of any parallel nervous activity, this effect certainly cannot be aroused unless the splanchnic fibers themselves have been previously stimulated. When the splanchnic fibers are no longer excitable because of an anatomical lesion the adrenal effect never appears.

Digitized by Google

This relationship is of particular interest with respect to the appearance of Traube-Hering waves. These have been noticed by all observers in the downward course of the final fall of blood pressure in the anemic response. Whether nervous centers are no longer excitable to sensory stimulation and whether or not the output of adrenalin is a factor concerned, remains to be tested. It must, however, be borne in mind that such a condition as this where Traube-Hering waves have been elicited, is preceded by an intense activity of the splanchnic outflow as stimulated by the medullary cells.

In the disturbance of the internal medium which the excessive concentration of carbon dioxide in the occluded cerebral vessels brings with it, the increased rate of flow is carried out and maintained by the vascular musculature, and some product of adrenal activity probably makes possible the maintenance of the increased impetus given the blood flow through such a prolonged period of time. However, it is only by virtue of the neurones within the central nervous system that the response is initiated, that it is regulated by changes in the cardiac musculature, and finally that the response is carried out as an integrated whole. The retention of a constant tension of carbon dioxide in the blood by means of an adaptive blood vascular reaction, is therefore mediated in the mammal through its higher central nervous organization, particularly the cells within the medulla oblongata.

To Prof. F. H. Pike the writer is greatly indebted for suggestions, advice and criticism, extended throughout the research.

CONCLUSIONS

1. The nerves of the heart are not essential either for the activation or for the persistence of the characteristic pressor phenomena of the anemic rise.

2. In the early stages of cerebral occlusion the cardiac innervation functions as a check on the rapid rise of blood pressure. In this moderating action, accelerators as well as vagi are involved, since on excision of the stellate ganglia, the vagi alone are unable to prevent an abrupt and steep rise of pressure.

3. The activation and maintenance of the vascular response under cerebral occlusion is controlled essentially by the splanchnic nerves.

4. Differential section in various regions of the splanchnic outflow influences the level of the arterial blood pressure. The extent to which the pressure falls on section is an approximate index of the degree to which the anemic rise will be compromised by the lesion.

Digitized by Google

5. It is impossible to influence the vascular response to anemia by indiscriminate sections within the splanchnic outflow. In order definitely to abolish the response, it is necessary to section either sufficiently far out in the periphery, or sufficiently high up in the spinal cord to interrupt completely the continuity between the medulla and the coeliac ganglion.

6. The level at which the fibers of the splanchnic system leave the spinal cord varies in different individuals. The greatest number of fibers leave the cord in the lower thoracic, especially in the region of the 6th to 8th thoracic. However, constrictor fibers to the splanchnic nerves leave the cord throughout the higher levels of the thoracic cord. In certain individuals, fibers leaving as high as the 2nd and 3rd thoracic will maintain an appreciable level of blood pressure and activate a significant anemic response.

7. Cerebral occlusion, carried out in repeated succession, is borne indefinitely (as many as 18 times) in intact animals. The occlusion time is in no way curtailed and the anemic increment of blood pressure only slightly diminished.

8. The curve of the anemic rise under repeated cerebral occlusion becomes dissociated into two distinct parts after eight or ten successive occlusions have been inflicted.

9. The long-continued maintenance of blood pressure at an extremely high level, characteristic of the anemic rise, is no longer possible after any gross interference with the supply of some product of adrenal activity.

10. An increased liberation of adrenalin under extreme splanchnic stimulation cannot be demonstrated as necessary for the characteristic contour of the anemic rise. This appears dependent on the amount of circulating adrenalin.

11. An increased availability of some product of adrenal activity appears demonstrable in intact animals under extreme splanchnic stimulation, after eight or ten successive occlusions have been inflicted.

12. Survival after ligation of the adrenal glands may be reduced to 1 or 2 hours, when the animal is subjected to successive repeated cerebral occlusions. A complete failure of vasomotor tone seems demonstrable in these animals.

13. The response of the splanchnic nerves is dependent for its release on conditions of functional activity within the brain stem.

14. The vasomotor responses initiated by the splanchnic nerves of the sympathetic nervous system are comparable with skeletal responses

Digitized by Google

dependent on the higher nervous levels, in respect to their complete dependence on these levels of the central nervous axis.

BIBLIOGRAPHY

(1) Stenson: Element Myelogiae specimen, etc. Amsterdam, 1667, 109.
(2) Swammerdam: Tractatus de respiratione, Lugd. Batav., 1687, 61.
(3) Magendie and Poiseuille: Leçons sur les phenomenes physiques de la vie 1837, t. III.
(4) Cooper: Guy's Hospital Reports, 1838.
(5) Batelli: Journ. Physiol. et. path. gen., 1900, ii, 443.
(6) Hill: Physiology and pathology of the cerebral circulation, London, 1896.
(7) Kussmaul and Tenner: Moleschott's Untersich. z. Naturlehre, 1857, iii, 1.
(8) Brown-Sequard: Compt. rend. de l'Acad. des sciences, 1851, xxvii, 83.
(9) Brown-Sequard: Journ. phys. de l'homme et des animaux, 1858, i, 117.
(10) Vulpian: Gazette hebd. de med. et chir., 1861, viii, 865.
(11) Schiffer: Centrlbl. f. d. med. Wis., 1869, 579 and 593.
(12) Cited by Luciani: Human physiology (transl. by Welby) 1911, i, 444.
(13) Cited by Hill: Mechanism of the circulation, Schäfer's Textbook, 1900, 136.
(14) Mayer: Sitzungsb. Wiener Akad. Wiss., 1874, Abt. 3, Bd. lxxiii, 85.
(15) Mayer: Medicin. Centrlbl., 1878, 579, 594.
(16) Couty: Arch. physiol. norm. et path., 1876, ser. 2, iii, 665.
(17) Goltz: Arch. path. u. Anat., 1864, xxix, 403.
(18) Goltz: Arch. f. d. gesammt. Physiol., 1874, viii, 3, 485.
(19) Vulpian: Leçons sur les vaso-moteurs, 1876, ii, 290.
(20) Schlesinger: Jahresb. f. d. gesammt. Med., 1874, i, 272.
(21) Kowalewsky and Adamük: Centrlbl. f. d. Med. Wis., 1868, 582.
(22) Bochefontaine: Arch. physiol., 1876, 142, quoted by Couty, loc. cit., 733.
(23) Mayer: Sitzungsb., Wiener Akad. d. Wis., 1879, Abt. 3, lxxix, 111.
(24) Konow and Stenbeck: Skand. Arch. f. Physiol., 1889, i, 407.
(25) Landergren: Skand. Arch. f. Physiol., 1897, vii, 1.
(26) Pike: Quart. Journ. Exper. Physiol., 1913, vii, 1.
(27) Langley: Journ. Physiol., 1919, xx, 236.
(28) Stewart, Guthrie, Burns and Pike: Journ. Exper. Med., 1906, viii, 289
(29) Stewart and Pike: This Journal, 1907, xix, 328.
(30) Stewart and Pike: This Journal, 1907, xx, 61.
(31) Pike, Guthrie and Stewart: Journ. Exper. Med., 1908, x, 371, 490.
(32) Pike, Guthrie and Stewart: This Journal, 1908, xxi, 359.
(33) Pike, Guthrie and Stewart: This Journal, 1908, xxii, 51.
(34) Pike: This Journal, 1909, xxiv, 124.
(35) Pike: This Journal, 1912, xxx, 436.
(36) Yates: This Journal, 1921, lvii, 68.
(37) Bethe: Ergebn. d. Physiol., 1906, 250.
(38) Hill and Flack: Journ. Physiol., 1908, xxxvii, 77.
(39) Hasselbach: Biochem. Zeitschr., 1912, xlvi, 403.
(40) Pike, Coombs and Hastings: Proc. Soc. Exper. Biol. and Med., xvi, 49.

Digitized by Google

(41) Hastings, Coombs and Pike: This Journal, 1921, lvii, 104.
(42) Mathison: Jour. Physiol., 1910, xli, 416.
(43) Mathison: Jour. Physiol., 1911, xlii, 283.
(44) Pike and Scott: Amer. Natur., 1915, xlix, 321.
(45) Bernard: Leçons Sur Les liquides de l'organisme, 1858, i, 200.
(46) Bezold: Unters. ueber die Innerv. des Herzens, Leipzig, 1863.
(47) Ludwig and Thiry: Sitzungsb. kais. Akad. Wis., 1864, xiv, 442.
(48) Ludwig and Cyon: Ber. Sachs Ges. Wis. Math. phys. cl., 1866, 318.
(49) Bezold and Bever: Unters. a. d. physiol. Lab. z. Wurzburg., 1867, i, 314.
(50) Cyon and Cyon: Arch. anat. u. physiol., 1857, 389.
(51) Mall: Arch. f. Anat. u. Physiol., 1892, 409.
(52) von Basch: Ber. Kais. Sächs Gesel. Wiss. Math. phys. cl., 1875, 413.
(53) Edwards: This Journal, 1914, xxxv, 15.
(54) von Anrep: Jour. Physiol., 1912, xlv, 307.
(55) Cathcart and Clark: Journ. Physiol., 1914, xlix, 301.
(56) Nawalichin: Centrlbl. Med. Wis., 1870, 483.
(57) Cited by Tigerstedt: Ergebn. d. Physiol., 1906, vi, 265.
(58) Erlanger: Proc. Amer. Physiol. Soc., This Journal, 1904, x, p. xiv.
(59) Hill: Schaefer's Textbook, 1900, 57.
(60) Wickwire: This Journal, 1920, liii, 355.
(61) Mosso: L'Impartialle xii, 1872.
(62) Hunt: Journ. Exper. Med., 1897, ii, 2.
(63) Stewart and Rogoff: This Journal, 1920, lii, 304.
(64) Langley: Schäfer's Textbook, 1900, 462.
(65) Ranson and Billingsley: Journ. Comp. Neurol., 1918, xxix, 405.
(66) Sherrington: Mammalian physiology, 1919, Oxford, 41.
(67) Bayliss: Journ. Physiol., 1893, xiv, 303.
(68) Oliver and Schäfer: Journ. Physiol., 1895, xviii, 230.
(69) Biedl: Arch. f. d. gesammt. Physiol., 1897, lxvii, 433.
(70) Dreyer: This Journal, 1897, ii, 203.
(71) Lewandowsky: Arch. f. Anat. u. Physiol., 1899, 360.
(72) Langley: Journ. Physiol., 1907, xxvii, 237.
(73) Elliott: Journ. Physiol., 1905, xxxii, 401.
(74) Elliott: Journ. Physiol., 1913, xlvi, 285.
(75) Elliott: Journ. Physiol., 1912, xliv, 375.
(76) Cannon and de la Paz: This Journal, 1911, xxviii, 64.
(77) Hoskins: Journ. Pharm. Exper. Therap., 1911, iii, 93.
(78) Folin, Cannon and Denis: Journ. Biol. Chem., 1913, xiii, 477.
(79) Stewart: Journ. Exper. Med., 1911, xiv, 377.
(80) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1916, viii, 479.
(81) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, ix, 393.
(82) Rogoff: Journ. Lab. Clin. Med., 1918, iii, 2.
(83) Cannon and Hoskins: This Journal, 1911, xxix, 274.
(84) Cannon: This Journal, 1914, xxxiii, 356.
(85) Cannon: Bodily changes in pain, hunger, fear and rage, New York, 1915.
(86) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, x, 49.
(87) Stewart and Rogoff: This Journal, 1917, xliv, 149.
(88) Stewart and Rogoff: This Journal, 1918, xlvi, 93.

Digitized by Google

(89) BURTON-OPITZ: Quart. Journ. Exper. Physiol., 1912, v, 82.
(90) BURTON-OPITZ: Quart. Journ. Exper. Physiol., 1912, v, 309.
(91) STEWART AND ROGOFF: Journ. Exper. Med., 1916, xxiv, 709.
(92) GLEY AND QUINQUAUD: Journ. Physiol. et Path. gen., 1918, xvii, 807.
(93) TSCHEBOKSAROFF: Arch. f. d. gesammt. Physiol., 1911, cxxxvii, 59.
(94) HOSKINS AND MCCLURE: Arch. Int. Med., 1912, x, 343.
(95) CYBULSKI: Wien. Med. Wochenschr., 1896, xlvi, 6.
(96) GLEY AND QUINQUAUD: Compt. rend. soc. biol., 1917.
(97) CZUBALSKI: Centrbl. Physiol., 1913, xxvii, 580.
(98) ELLIOTT: Journ. Physiol., 1914, xlix, 38.
(99) CUSHING: Grenzgebiet Med. u. Chir.—Physiol. Anat. Beob., 1902.
(100) PORTER, MARKS AND SWIFT: This Journal, 1908, xx, 40.
(101) PORTER: Harvey Lectures, 1906–7.
(102) HOSKINS AND MCCLURE: This Journal, 1912, xxx, 192.
(103) YOUNG AND LEHMAN: Journ. Physiol., 1908, xxxvii, p. liv.
(104) BURTON-OPITZ AND EDWARDS: This Journal, 1916, xli, 91.
(105) JOHANNSEN: Arch. Anat. u. Physiol., 1891, 103.
(106) LEHNDORFF: Arch. Anat. u. physiol., 1908, 362.
(107) PEARLMAN AND VINCENT: Endocrinology, 1919, iii, 121.
(108) LUCAS: Conduction of the nervous impulse, London, 1920, 70.
(109) HULTGREN AND ANDERSEN: Skand. Arch. Physiol., 1899, ix, 73.
(110) GAUTRELET AND THOMAS: Compt. rend. soc. de. biol., 1909, 388.
(111) HOSKINS: This Journal, 1915, xxxvi, 423.
(112) BAZETT: Journ. Physiol., 1920, liii, 320.
(113) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1917, xi, 1.
(114) STEWART AND ROGOFF: This Journal, 1919, xlviii, 397.
(115) BICHAT: Anatomie Generale, 1830, i, 285.
(116) VOLKMANN: Wagner's Handworterbuch, 1879.
(117) GOLTZ AND EWALD: Arch. f. d. gesammt. Physiol., 1896, lxiii, 362.
(118) ECKHARD: Hermann's Handb., 1879, ii, 2.
(119) MAYER: Hermann's Handb., 1879, ii, 275.
(120) GASKELL: Involuntary nervous system, London, 1916.
(121) SHERRINGTON: The integrative action of the nervous system, New Haven, 1906, 243, 308.
(122) RANSOM: Nervous system, Philadelphia, 1921.
(123) PIKE: Journ. Comp. Neurol., 1918, xxix, 485.
(124) SHERRINGTON: Proc. Roy. Soc., lxvi.

Digitized by Google

THE RÔLE OF THE SODIUM AND THE CARBONATE IONS AND OF THE CHANGE IN THE SODIUM-CALCIUM RATIO IN THE CONTRACTION OF THE ISOLATED DUODENAL SEGMENT OF THE ALBINO RAT

F. S. HAMMETT AND J. E. NOWREY, Jr.

From The Wistar Institute of Anatomy and Biology, Philadelphia

Received for publication November 10, 1921

Previous studies have shown that the isolated duodenal segment of the adult, male, unexcited albino rat when suspended in oxygenated Tyrode's solution at body temperature contracts when small amounts (0.1 to 0.4 cc.) of tenth molecular sodium carbonate solution are added to the surrounding liquid (1), (2).

Two questions that naturally arise as a result of this reaction are: What is the intestinal mechanism stimulated by the sodium carbonate? and—What component of, or condition occasioned by the addition of sodium carbonate to, the surrounding Tyrode's solution furnishes the stimulus to contraction?

In a previous publication it has been shown that the contraction in question results from a stimulation of the musculature by those nerve elements normally concerned in contraction (3). This neural stimulation is set into activity by one or more of the components of, or conditions occasioned by the addition of the sodium carbonate to, the Tyrode's solution bathing the segment.

This addition causes an increase in the concentration of the sodium ions and of the carbonate ions. There is also an increase in the sodium-calcium ratio and in the hydroxyl ion concentration. We will consider the first three of these possibilities in this report and leave the last for the succeeding paper.

In general the procedure in these experiments was the same as that already described (2), (3).

Now if the shortening of the segment is due to a stimulation due to the increase in the sodium ions, the addition to the solution of an amount of these ions in the form of sodium chloride equivalent to the amount of sodium ions added as sodium carbonate, should produce the same height of contraction as results from the latter stimulus.

Digitized by Google

If the contraction following the sodium carbonate application is a result of an increase in the sodium-calcium ratio, there is no reason why the equivalent increase of this ratio should not produce the same result as when the ratio is increased by sodium carbonate. This possibility is to be considered since in the opinion of Loeb (4) changes in the concentrations of antagonistic ions or salts are the means by which stimulations are brought about, and this opinion is supported not only by his own studies but also by those of Osterhout (5), Lillie (6), Benda (7) and others.

Since, however, the addition of 0.4 cc. of a tenth molecular solution of sodium carbonate to the 4.0 cc. of Tyrode's solution occasionally gives rise to a perceptible precipitation of calcium salt, the use of that salt as a standardizing reagent in these tests is obviously inadvisable. Instead of sodium carbonate, then, we have used sodium bicarbonate as the standardizing substance, for when 0.4 cc. of a fifth molecular solution of this compound is added to 4.0 cc. of Tyrode's solution no visible evidence of calcium precipitation occurs during the test, and a characteristic shortening of the segment is obtained, as already shown by Young (8).

With these facts in mind the effect of 0.4 cc. of a fifth molecular solution of sodium chloride on the duodenal segment of the albino rat standardized with 0.4 cc. of a fifth molecular solution of sodium bicarbonate was investigated according to the procedure employed in the earlier studies (11), (3).

The results of such a test are shown in the accompanying tracing.

It is evident that the addition of an amount of sodium in the form of sodium chloride to the Tyrode's solution equal to that added in the form of sodium bicarbonate failed to produce the characteristic contraction brought about by the latter compound. This fact allows us to conclude that neither the increased sodium ions as such, nor the increased sodium-calcium ratio is the significant factor in causing the contraction.

The objection may be raised that the addition of this amount of sodium chloride to the 4 cc. of Tyrode's solution is too small to seriously affect the sodium-calcium ratio. If we grant the objection we can nevertheless counter with the observation that the same or even a lesser increase in the sodium-calcium ratio when brought about by sodium in the form of sodium bicarbonate causes a contraction of the segment and our conclusion is still justified.

For a substance to act as a stimulant to contraction it must reach

Digitized by Google

BOSTON MEDICAL
MAY 13 1922
LIBRARY

the mechanism on which it is to act. In other words, the tissue cells must be permeable to the effective agent. The importance of the permeability of the cell has been recognized since the time of Asclepiades (10) who based his system of medicine on the belief that health and disease depend more or less on the relation existing between the

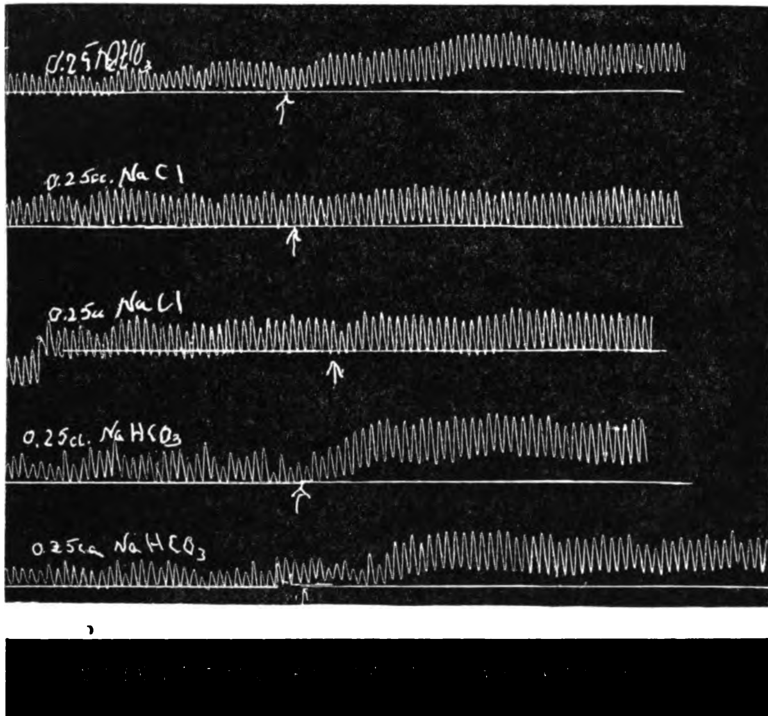


Fig. 1. The effect of fifth molecular sodium chloride solution on the isolated duodenal segment of the albino rat. The first two tracings show the effects on the segment of 0.4 cc. of a fifth molecular solution of sodium bicarbonate. The third and fourth tracings were made when there had been added 0.4 cc. of a fifth molecular solution of sodium chloride. The fifth tracing was made with sodium bicarbonate.

molecules and the pores through which they must pass. The studies of Loeb (11) and Osterhout (12) have quite definitely shown that permeability is favorably influenced by sodium ions and unfavorably by calcium ions. It may be that the increase in the sodium ions as such or through the increase in the sodium-calcium ratio facilitates, as it were, the admission of the exciting ions to the stimulated mechanism. Thus the sodium ions can be considered as participating

Digitized by Google

in the reaction, but they can not in any way be considered as the agents setting the neural mechanism into that activity which results in a shortening of the duodenal segment. This belief is directly opposed to that of Loeb (13) with respect to the hydroxyl ion effect.

As far as the carbonate ions are concerned, they can hardly be considered as an important determinant of the response since one of us has shown that the duodenal segment reacts to all appearances to sodium hydroxide just as it does to sodium carbonate (14). Although the addition of sodium hydroxide solution to Tyrode's solution is presumably accompanied by the reaction

$$NaOH + NaHCO_3 = Na_2CO_3 + H_2O$$

this can have but little if any effect upon the concentration of the carbonate ions when there is taken into consideration the relative ionization of the dissociation products of sodium carbonate.

CONCLUSION

The contraction of the isolated duodenal segment of the albino rat which follows the addition of tenth molecular carbonate solution to the oxygenated Tyrode's solution in which the segment is suspended, is due neither to the increase in the sodium ions, nor in the sodium-calcium ratio, nor in the carbonate ions. It is true that the increase in the sodium ions may participate in the effect by increasing the permeability of the tissue for the agent initiating the reaction, but this increase in permeability can not be considered as the primary cause of the contraction.

BIBLIOGRAPHY

(1) Hatai and Hammett: This Journal, 1920, liii, 312.
(2) Hammett: This Journal, 1921, lv, 404.
(3) Hammett: This Journal, 1921, lv, 414.
(4) Loeb: Proc. Nat. Acad. Sci., 1915, l, 438.
(5) Osterhout: Science, 1916, N.S., xliv, 395.
(6) Lillie: This Journal, 1909, xxiv, 14.
(7) Benda: Z. Biol., lxiii, 11.
(8) Young: Quart. Journ. Exper. Physiol., 1915, viii, 347.
(9) Hammett and Tokuda: This Journal, 1921, lvi, 380.
(10) Cabanis: An essay on the certainty of medicine. Transl. by R. La Roche, Philadelphia, 1823.
(11) Loeb: Biochem. Zeitschr., 1912, xlvii, 127.
(12) Osterhout: Science, 1912, N.S., xxxv, 112; xxxvi, 350.
(13) Loeb: Studies in general physiology, Chicago, 1905, ii, 518.
(14) Hammett: This Journal, 1921, lvi, 385.

Digitized by Google

THE RÔLE OF THE CHANGE IN HYDROGEN-ION CONCENTRATION IN THE MOTOR ACTIVITIES OF THE SMALL INTESTINE

FREDERICK S. HAMMETT[1]

From The Wistar Institute of Anatomy and Biology, Philadelphia

Received for publication November 10, 1921

In the preceding paper (1) it was shown that the contraction of the isolated intestinal segment of the albino rat suspended in oxygenated Tyrode's solution at body temperature on the addition of fifth molecular sodium bicarbonate to the surrounding fluid, is not due to the increase in either the sodium ions, the sodium-calcium ratio or the carbonate ions.

In this paper there will be presented evidence for the view that the effective agent for the observed contraction is the increase in the hydroxyl-ion concentration of the fluid in which the segment is suspended following the addition of the alkaline compound used.

In view of this result and other correlated data the probable relation between the changes in hydrogen-ion concentration of the material coming in contact with the intestine and intestinal movements will be discussed.

In order to determine whether or not the observed contraction of the isolated intestinal segment is due to the increase in hydroxyl-ion concentration of the Tyrode's solutions on the addition of tenth molecular sodium carbonate there was prepared a solution of sodium hydroxide which would give the same hydroxyl-ion concentration when added in the same amount to equal quantities (4 cc.) of Tyrode's solution as is given by the tenth molecular sodium carbonate solution. Using the indicator method it was found that the addition of 0.2 cc. of a solution of sodium hydroxide of approximately twentieth molecular concentration to the 4 cc. of Tyrode's solution gave a pH between 9.4 and 9.8. The addition of 0.2 cc. of a tenth molecular solution of sodium carbonate to 4 cc. of Tyrode's solution gave the same pH. A comparison of the

[1] Much of the experimentation reported in this paper was carried on by Mr. J. E. Nowrey, Jr., to whom the author wishes to express his thanks.

Digitized by Google

two resultant colored solutions in the Duboscq colorimeter showed an agreement in color depth that was satisfactory.

Intestinal segments were prepared as described in a previous publication (2) and their responses to the addition of equal amounts of these two solutions to equal amounts of Tyrode's solution in which they were suspended were recorded as usual.

It was found that practically the same degree of contraction was obtained with the sodium hydroxide solution as with the sodium carbonate solution. This is shown in the accompanying tracing. It allows of no other conclusion than that *the stimulus to contraction is the increase in the hydroxyl-ion concentration of the liquid in which the segment is suspended.*

This result is also a confirmation of the findings of the preceding (1) paper, because on the one hand the concentration of the sodium ions in the sodium hydroxide solution used was less than a fourth of that of the sodium carbonate solution; and on the other, the carbonate-ion concentrations were in no sense comparable.

This result may also serve in part, at least, to explain on the basis of the change in hydroxyl-ion concentration the stimulating effect of various alkaline salts on intestinal contraction observed by Salant, Mitchell and Schwarze (3), Salant and Schwartze (4), Alvarez (5), Starkenstein (6) and others.

Before discussing the indications of the foregoing observations a brief review of the studies leading up to the ideas about to be expressed is not out of place.

It was observed by Hatai and Hammett (7) that the isolated duodenal segment of the albino rat, when suspended in oxygenated Tyrode's solution at body temperature, responds by a contraction when small amounts of tenth molecular sodium carbonate solution are added to the liquid surrounding the segment. It was found that this reaction was reversed, i.e., that a relaxation occurred *a*, when the animal had been excited or angered previous to the removal of the segment for testing; *b*, when the splanchnics were electrically stimulated in the dead animal before removal of the segment; and *c*, during periods of physiological or emotional instability such as occur in young animals or menstruating females. It was also noticed that approximately equal degrees of contraction followed the application of equal amounts of the carbonate solution to segments from the normal, adult, unexcited male rat. Further investigations established the fact that segments from such animals could serve as accurate testing material for the quantitative

Digitized by Google

comparison of tissue extracts and other substances within reasonable limits when sodium carbonate is used as the standardizing reagent (2). These observations gave a basis for testing the intestinal contracting ability of thyroid extracts from the glands of rats of different ages (8) and the action of thyroxin on the isolated intestinal segment (9). The next study was devoted to a determination of the intestinal mech-

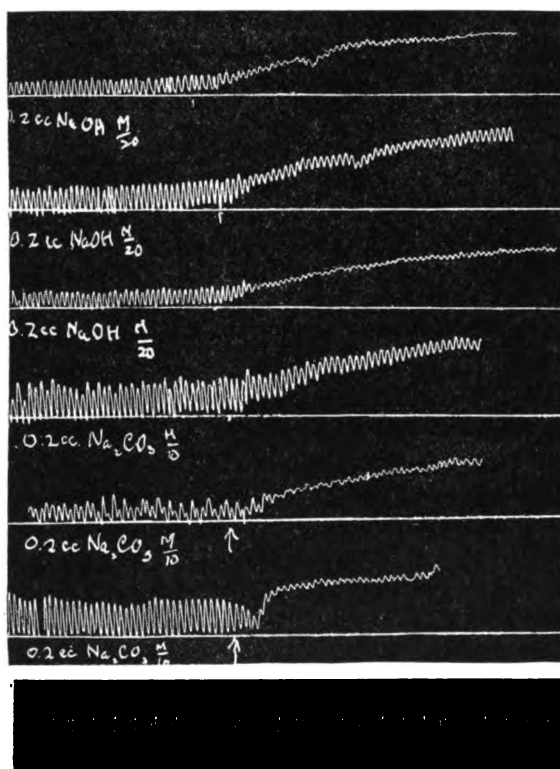


Fig. 1. Effect of equal H-ion concentrations from NaOH and from Na_2CO_3 on the degree of contraction of the isolated intestinal segment. Time in 5 seconds.

anism primarily stimulated by the carbonate (10). It was found that the neural mechanism is primarily involved in the phenomena recorded. This led us to realize that a determination of the agent or condition effective in producing the contraction might throw some light upon the nature of the factors directly regulating or controlling intestinal movements. The preceding paper (1) and the evidence presented in this

Digitized by Google

one have shown that neither the increase in the sodium ions, nor the sodium-calcium ratio, nor the carbonate-ion concentration is the effective agent, but that the stimulus to contraction is the increased hydroxyl-ion concentration derived from the dissociation of the added sodium carbonate.

We are at once confronted with the question as to how far we are justified in utilizing the findings of these experiments *in vitro* for an interpretation of the probable control or influence of changes in hydrogen-ion concentration on the motor activities of the intestine. The direct transfer of these observations must be made with caution and with reservations explicit or implied.

Although Cannon (11), Gaskell (12), Alvarez (13) and others have recognized the probability that the motor activities of the intestinal musculature are modified by chemical factors, nevertheless the interpretations of these phenomena, with the exception of the latter investigator, have been almost entirely based on the idea of a predominantly neural control as developed from the studies of Bayliss and Starling (14), (15), Magnus (16) and others. Nevertheless it is undeniable that the initiation of every stimulus which is carried to the muscle tissue by the nerve fibers is, in the last analysis, due to a chemical change. That the ultimate explanation will be based on energy changes is obvious and has been recently emphasized for biological processes by J. Traube (17).

It is not necessary to go into any detailed exposition of the proved and probable chemical sources of stimuli produced by the organism affecting either directly or indirectly the motor activities of the intestine. With no intention of minimizing the importance of their rôle, it is felt that the studies of Henderson (18), Michaelis (19), Loeb (20), Moore (21), Clark (22), Lillie (23), Osterhout (24) and others on the exquisite sensitivity of living cells to change in hydrogen-ion concentration give ample evidence of the predominant part which this type of chemical change plays in the phenomena of stimulation in general and of nerve stimulation in particular.

We have shown in this and previous studies that the intestinal segment shortens or contracts when an increase in the pH or hydroxyl-ion concentration occurs in the solution with which it is in contact. It has also been shown that this effect is produced by transmission of the stimulation through the neural mechanism of the segment. That other compounds which on dissociation in solution give rise to an alkaline reaction produce the same effect has been alluded to before.

Digitized by Google

On the other hand, Young (25) has definitely shown that an excess of hydrogen ions produces just the opposite effect, namely, a relaxation, when added to the solution in which the segment is suspended. The experiments of Alvarez (26) support these observations in their general aspect.

It is thus evident that changes in the hydrogen-ion concentration of the medium with which the intestine comes in contact have a marked influence upon its motor activities; an excess of hydroxyl-ions causing a contraction, an excess of hydrogen ions a relaxation.

That changes in the hydrogen-ion concentration of the intestinal contents are bound to occur is evident from the observations of Busch (27), Auerbach and Pick (28), McClendon and his collaborators (29) and others that the succus entericus is normally alkaline in reaction, and the finding of Foa (20) that the pancreatic juice is similarly constituted, when correlated with the observations of Moore and Bergin (31) and McClendon and his collaborators (32) that the reaction of the intestinal contents on removal after eating is normally acid. In addition there is the well-known fact of the passage of the hydrochloric acid of the gastric juice into the duodenum during the process of digestion. It is therefore certain that there takes place in the intestine a continuous swinging to and fro between alkalinity and acidity of the reaction of the material coming in contact with the intestinal wall.

During this play back and forth between these oppositely acting agents of stimulation there must arise innumerable opportunities for these changes to be transmitted by diffusion into the intestinal wall and into contact with the neural mechanisms concerned in the motor activity and there set into play now one, now the other type of response, according to the specific mechanism stimulated. That such may conceivably occur appears from the observations of Loeb and Wasteneys (33) that weak alkalies, and of Crozier (34) that weak acids penetrate the cellular membranes with extraordinary facility, and the findings of Langley (35) that it is the nature of the nerve endings and not the impulses carried by the nerves that determine the nature of the response.

We do not wish to be understood as holding that the stimulation to contraction or to relaxation is due to a direct contact of an excess of hydroxyl ions or of hydrogen ions derived immediately from the changes in reaction of the intestinal contents with the neural elements directing the motor activities of the intestine. While this may be so, it appears more probable that the penetration of the cell layers immediately in contact with the intestinal contents by varying hydroxyl- or hydrogen-

Digitized by Google

ion concentrations which set up their characteristic effects, is followed by a transmission of these effects through a local and generally limited zone until neutralized by meeting zones of opposite reaction. In this spread of the effect primarily induced by changes in hydrogen-ion concentration of the intestinal contents in contact with any given spot of the intestine at any given moment and which changes from moment to moment, there is encountered a portion of the neural mechanism of the intestine and the appropriate response is elicited depending upon the nature of the stimulus. Further analysis will probably reveal that this spreading effect is of the nature of physico-chemical changes in surface charges or potential following changes in ionization.

Inasmuch as little if anything is accurately known of the precise rôle of the neural supply to the intestinal musculature in the various motor activities exhibited in katastalsis, anastalsis, segmentation and the myenteric reflex it would be wasted speculation to attempt an analysis of the specific rôle of the changes in hydrogen-ion concentration in these phenomena.

Nevertheless it may be assumed from what is known of the general nature of the response elicited by stimuli coming in over the vagal or splanchnic pathways and the known effect of hydrogen-ion or hydroxyl-ion excess on intestinal movement, that the nerve endings of the vagal mechanism are affected by a predominant hydroxyl-ion concentration while the splanchnic endings are affected by a predominant hydrogen-ion concentration. This assumption, however, does not facilitate, in the present state of our knowledge, an interpretation of the phenomena of specific intestinal movements. Until information is to be had of the finer details of the neural direction of the intestinal motor activities, and until the changes in reaction of the fluids in their passage through the intestinal wall and their effect upon the protoplasm of the intestinal cells are known, any application of the above assumption is precluded.

This lack of information, however, does not destroy the validity of the idea that changes in the hydrogen- and hydroxyl-ion concentration of the material coming in contact with the intestine are important participants in the regulation and control of the intestinal motor activities.

We are not to be understood as taking the stand that the motor activities of the intestine are solely dominated by the effects of a local stimulation of the intestinal neural mechanisms by changes taking place in the hydrogen-ion concentration of the intestinal contents. Nor do we hold that changes in the hydrogen-ion concentration at any one point in the system are the sole determinants of the nature of the response

Digitized by Google

elicited. For there is ample evidence that a strictly neurogenic influence, in the sense of a central participation, may at times supersede a local chemical influence. The opposite may also occur. But we believe that in the normal undisturbed organism during digestion the changes in the hydrogen-ion concentration of the intestinal contents determine the intestinal motor activities which are superintended and directed by the intestinal neural mechanisms.

BIBLIOGRAPHY

(1) Hammett and Nowrey: This Journal, 1922, lx, 48.
(2) Hammett: This Journal, 1921, lv, 404.
(3) Salant, Mitchell and Schwartze: Journ. Pharm. Exper. Therap., 1916–1917, ix, 511.
(4) Salant and Schwartze: Journ. Pharm. Exper. Therap., 1916–1917, ix, 497.
(5) Alvarez: This Journal, 1918, xlvi, 554.
(6) Starkenstein: Biochem. Zeitschr., 1920, cvi, 172.
(7) Hatai and Hammett: This Journal, 1921, liii, 312.
(8) Hammett and Tokuda: This Journal, 1921, lvi, 380.
(9) Hammett: This Journal, 1921, lvi, 386.
(10) Hammett: This Journal, 1921, lv, 414.
(11) Cannon: This Journal, 1912, xxx, 114.
(12) Gaskell: The involuntary nervous system, London, 1920.
(13) Alvarez: This Journal, 1918, xlvi, 186.
(14) Bayliss and Starling: Journ. Physiol., 1899, xxiv, 99.
(15) Bayliss and Starling: Journ. Physiol., 1900, xxvi, 125.
(16) Magnus: Arch. f. d. gesammt. Physiol., 1904, cii, 123; 349; 525.
(17) Traube: Biochem. Zeitschr., 1921, cxx, 108.
(18) Henderson: The fitness of the environment, New York, 1913.
(19) Michaelis: Oppenheimer's Handb. d. Biochem., Erganzungbd., Jena, 1913, 10.
(20) Loeb: Arch. f. d. gesammt Physiol., 1897, lxix, 1.
(21) Moore: Journ. Physiol., 1920, liii, lvii.
(22) Clark: Journ. Physiol., 1913, xlvii, 66.
(23) Lillie: Journ. Biol. Chem., 1916, xxiv, 233.
(24) Osterhout: Journ. Biol. Chem., 1914, xix, 335.
(25) Young: Quart. Journ. Exper. Physiol., 1915, viii, 347.
(26) Alvarez: This Journal, 1918, xlvi, 554.
(27) Busch: Arch. f. path. Anat. u. Physiol., 1858, xiv, 140.
(28) Auerbach and Pick: Arb. f. Gsndsamte., 1912, xliii, 155.
(29) McClendon, Bissell, Lowe and Meyer: Journ. Amer. Med. Assoc., 1920, lxxv, 1638.
(30) Foa: Compt. rend. Soc. de Biol., 1905, lix, 867.
(31) Moore and Bergin: This Journal, 1900, iii, 316.
(32) McClendon, Shedlov and Karpman: Journ. Biol. Chem., 1918, xxxiv, 1.
(33) Loeb and Wasteneys: Journ. Biol. Chem., 1913, xiv, 355.
(34) Crozier: This Journal, 1918, xlv, 323.
(35) Langley: Journ. Physiol., 1898, xxiii, 240.

Digitized by Google

THE ELECTRICAL CONDUCTIVITY OF ANIMAL TISSUES UNDER NORMAL AND PATHOLOGICAL CONDITIONS

GEORGE W. CRILE, HELEN R. HOSMER AND AMY F. ROWLAND

The Cleveland Clinic, Cleveland, Ohio

Received for publication November 14, 1921

The researches of Lillie (1), Loeb (2), Osterhout (3), Mathews (4), McClendon (5), Hill (6), Lucas (7) and of many other biophysicists and chemists (8) indicate that the functions of the cells of living organisms are related to electrical processes; that the living cell, whether it exists alone or as an element in a complex organism, possesses a certain store of potential energy which is manifested by variations in polarity and by action currents; that variations in the permeability of the living cell to the electrically charged elements of the fluid which surrounds it parallel variations in irritability in response to stimulation; that factors which suspend or abolish irritability also suspend or abolish alterations in permeability.

Every activity of living tissue is accompanied by electrical currents; and many activities are also initiated by electrical currents. In fact, the work of the investigators referred to above shows how strong is the tendency to consider that vital processes depend upon electric energy, by means of which also the protoplasm is renewed, and the whole mechanism is constructed.

In view of this trend of physiological conceptions, the electrical properties of living protoplasm become of vital interest. As this interest extends, the need of definite quantitative data increases. The laws which govern the action of electrical forces in inorganic systems are known exactly. It is possible to calculate exactly how much heat, or what chemical change, or how much work will result from the passage of a current of known strength through a known resistance during a definite period of time.

The two independent factors, current and resistance respectively, depend upon the amount of available electrical energy and the constitution of the system in which its conversion into heat, or chemical change, or other type of work is to be accomplished.

Digitized by Google

The action of electrical energy in protoplasm, although all the conditions are far more complicated than in inorganic substances, is governed by the same laws. In protoplasm, as in inorganic matter, electrical currents will always choose the path over the lowest available resistance; and in protoplasm, as in inorganic matter, the current pays toll to the friction offered by the system through which it passes.

The facts already established regarding bio-electric currents are sufficient to indicate the importance of further investigation, especially along certain lines. For example: What is the range of the electric conductance of living tissue? How does that range compare with that of other electrical conductors? Is the range of conductance the same for all types of tissue, and in each tissue does it remain constant under all conditions? Is the electric conductance of each tissue a factor in the production of the activities of the organism, to which a fairly constant value can be assigned?

These are questions which occur at once to the most casual student of bio-electric problems, and the fact that the literature offers no clear answer is sufficient reason for a detached study of this subject.

During recent years various investigators have applied measurements of electrical conductivity to the determination of variations in the permeability of protoplasm under varying conditions. In particular the work of Osterhout, Lillie and Loeb along these lines is too well known to be more than mentioned here.

Other investigators have used conductivity measurements as a means for estimating the volume of the corpuscles in blood, for determining the H-ion concentration in body fluids, for measuring the variations in the conductivity of muscle which result from contraction. But none of these investigators have attempted to determine the specific conductance of any tissue.

Early in this century Galeotti (9) carried out a limited number of experiments for the purpose of studying the changes which occur at death. He utilized the tissues of dogs, rabbits, guinea pigs, frogs and turtles, making measurements at successive intervals after the removal of the tissue from the animal. In general, he observed a rapid decrease in conductivity during the first few minutes, followed by a more gradual decrease, which in some instances lasted for several hours. After reaching the minimum, which he considered marked the death point of the tissue, the conductivity began to rise, gradually at first, and then very rapidly until a very high value was reached.

Digitized by Google

His values for the normal conductivity of certain rabbit tissues may be of interest as compared with those observed in the work to be described later (tables 1 and 2).

For the most part Galeotti worked with the firmer tissues, sections of which he introduced directly between platinum electrodes which were clamped in place after the application of a greater or less degree of pressure. He used only a few animals of each species and does not

TABLE 1

Specific conductivity of certain rabbit tissues as determined by Galeotti. (Expressed in reciprocal ohms)

TEMPERATURE	LIVER	HEART	MUSCLE	
			Longitudinal	Transverse
12°C.			00182	00058
18°C.	000268	000799		000804
	00090			
	00053			
24°C.	000269			000789

TABLE 2

Comparison of the specific conductivity of rabbit blood before and after coagulation as determined by Galeotti. (Expressed in reciprocal ohms)

TEMPERATURE	BEFORE COAGULATION	AFTER COAGULATION
38°C.	00569	00566
	00679	00674
	00773	00771
	00539	00526
	00631	00590

state that he measured more than one sample of each tissue from any one animal.

The work to be described below was the direct outcome of preliminary measurements made by G. B. Obear of the Case School of Applied Science in the fall of 1917. In spite of inadequate apparatus and a limited number of observations, Doctor Obear's results indicated such consistent relative values of the specific conductivities of certain tissues, especially the cerebrum and cerebellum, as to encourage a further research under better conditions. In the fall of 1918, therefore, the research was continued.

Digitized by Google

Apparatus. The apparatus employed in this research includes that devised by Dr. E. W. Washburn and developed for the market by Leeds and Northrup. It consists of the typical Wheatstone bridge made up of a Kohlrausch slide wire and a resistance box of Curtis coils, with a telephone connected across the ends of the slide wire. Suitable capacities for tuning the circuit and for balancing the capacity of the conductivity cell were inserted in the current. A high frequency (1,000 cycles) alternating current was obtained from a constant speed high frequency generator located in another room. All measurements were made with the cell partially immersed in a constant temperature bath. A Freas bath of 300 liters capacity was used, supplemented by a specially constructed cover to minimize fluctuations of temperature, to maintain a sufficiently humid atmosphere, and to insure maintenance of the leads and upper part of the conductivity cell at a uniform temperature.

On account of faulty construction of the glass parts, the automatic temperature regulator for this bath has never worked satisfactorily, but by hand regulation of the lights it has not been difficult to keep the temperature constant within 0.1°C.

Washburn's recommendations in regard to magnetic shielding, grounding, etc., have been carefully observed.

Figure 1 shows the arrangement of this apparatus as used in this research.

Electrodes. As the early part of this work was done at a time when it was a patriotic duty to conserve platinum, in the preliminary experiments all types of tissue were measured in the same set of electrodes, though it is obvious that this is far from an ideal mode of procedure.

Sections of each tissue were packed into small glass tubes of various sizes, each of which was accurately ground to insure uniform dimensions throughout. The tubes were packed with a sufficient excess of material to procure a slight projection from each end, and were placed between thin platinum electrodes, reinforced by brass backings. Sufficient pressure was applied to bring the electrodes flush with the ends of the tubes, when the electrodes were firmly clamped into place. Great pains were taken to avoid air spaces within the tubes and to insure uniform contact of the tissues with the electrodes. This was not difficult with the softer tissues, such as brain and liver, but with tougher tissues such as muscle and thyroid it was impossible to exclude considerable error from imperfect contact and other variations. The effect of these faults is plainly evident in the greater variation in the conductance values obtained for the latter tissues.

Digitized by Google

The tubes used for the measurement of the conductivity of the brain, the liver and voluntary and involuntary muscle, were approximately 5 mm. in diameter and 5 mm. in length, while those used in the measurement of the adrenals and the thyroid were of the same length with a diameter of approximately 2.5 mm. Special hard rubber containers were devised for the spinal cord (fig. 2).

The conductance capacities or cell constants of these tubes were determined by repeated measurements of their conductance when filled

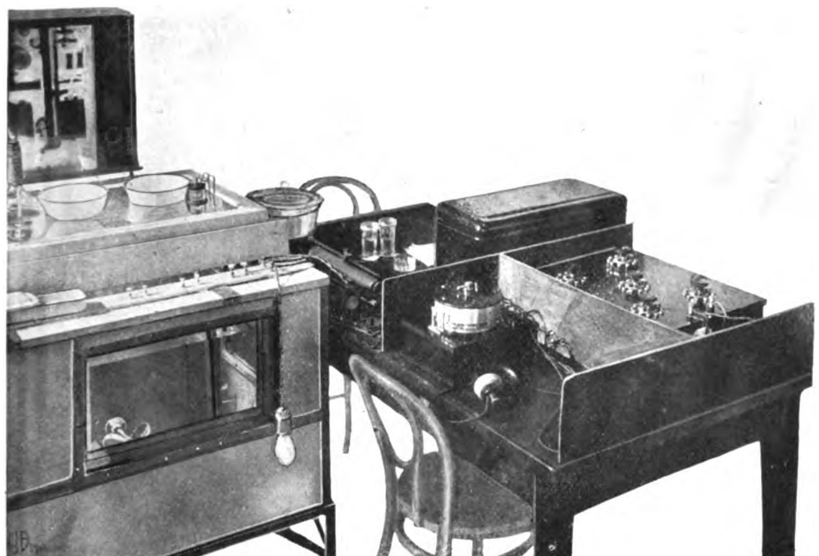


Fig. 1. Arrangement of apparatus for electrical conductivity research

with 0.01 NKCl, at the same temperature as that used for the tissue measurements.

In the later work larger tubes 1 cm. long × 1 cm. in diameter were used. These were only partially filled with tissue, and an upper electrode of the type shown in figure 3 was used. This electrode was 1 cm. in diameter and was pierced by slits. The tube was partially filled with closely packed material, and carefully placed in position on the lower electrode, after which the upper electrode was inserted within the tube and carried down until contact was made with the upper

Digitized by Google

surface of the tissue, care being taken, however, to avoid sufficient pressure to cause the extrusion of material through the slits. This upper electrode was then clamped in place and the distance between the two electrodes was accurately measured and recorded.

Every precaution was taken to avoid any undue pressure with the consequent reduction of the fluid content of the tissue and resultant

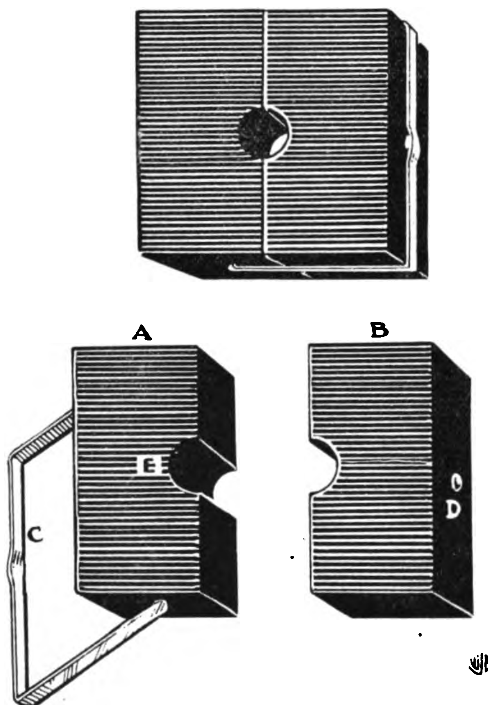


Fig. 2. Special container devised for making conductivity measurements of the spinal cord. *A* and *B* are blocks of hard rubber. The section of spinal cord is laid in the curved groove in one of these, the two pieces brought together and clamped by carrying the brass loop *C* over *D*.

lowering of the conductance, although this danger was lessened by the use of the pierced electrode. Various measurements of different types of tissue were made to determine the effect of varying the pressure, the results of which are illustrated by the groups of measurements shown in table 3. In each case the successive measurements were made upon the same sample of tissue, the distance between the electrodes,

Digitized by Google

and consequently the pressure, being changed between each two measurements. The results indicate that a greater error is to be feared from the application of too much pressure than from too light a contact.

The cell constants for the tubes used in the later experiments, as for those used in the earlier series, were determined by measurements with 0.01 NKCl with the electrodes at different distances apart, these

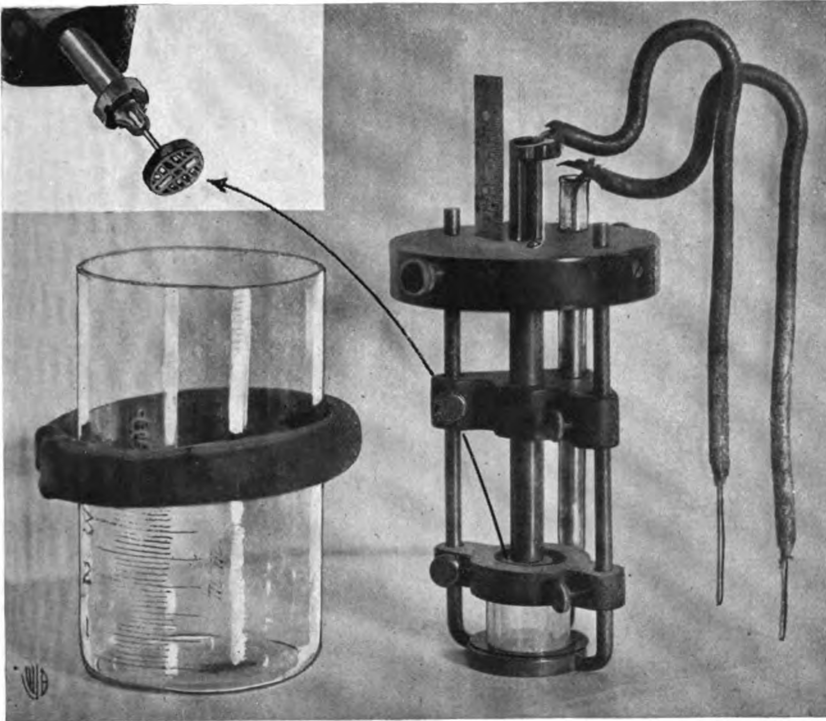


Fig. 3. Electrical conductivity cell and detailed drawing of upper electrode

results being plotted for convenient use in estimating the value of the tissue measurements.

The electrodes were carefully cleansed after each measurement and were replatinized at intervals with a very light coating of platinum black. The electrodes and holders were always placed in the bath long enough before use to insure the thorough warming up of the metal and glass parts, and during the insertion of tissue were exposed as little as possible to lower temperatures, and even then they were given

Digitized by Google

TABLE 3

Effect of variations in pressure upon the electrical conductivity of animal tissues

TISSUE	DISTANCE BETWEEN ELECTRODES	CONDUCTIVITY (EXPRESSED IN RECIPROCAL OHMS)
	cm	
Cerebellum		
Section 1	0.78	00133
	0.66	00129
	0 48	00114
Section 2	0.92	00148
	0 55	00128
Section 3	0.60	00140
	0.52	00136
	0 40	00123
Section 4	0.65	00115
	0.50	00108
	0 40	00105
Section 5	0 75	00121
	0 65	00116
	0 50	00116
Section 6	1.11	00158
	0.96	00151
	0.65	00128
Cerebrum		
Section 1	1.24	00183
	0.94	00167
	0.72	00159
Section 2	1.20	00183
	0.92	00170
	0 81	00169
Section 3	1.03	00172
	0.85	00164
	0.64	00145

an opportunity to reach the bath temperature before measurements were made. It was possible to exercise these precautions with but little loss of time by using two sets of electrodes and holders. All

Digitized by Google

measurements were either made at 39°C. or were corrected to that value from temperatures not over a degree removed from it.

Special points in technique. The object of this investigation being to determine the electric conductivity of animal tissues under conditions as nearly as possible identical with those existing in the living animal, every effort was made to keep the sections of each tissue always at a normal body temperature; to avoid any loss of moisture; and to measure each at the earliest possible moment after the death of the animal and the removal of the tissue from the body.

A series of measurements was made to determine the onset of post-mortem changes in the different tissues as evidenced by changes in the electric conductivity.

TABLE 4

Conductivity measurements made at varying periods after death to determine onset of post-mortem changes

(Conductivity expressed in reciprocal ohms)

	PERIOD AFTER DEATH							
	Immediate	½ hour	1 hour	1½ hours	2 hours	2½ hours	3 hours	4 hours
Cerebellum	00139	00140	00140	00148	00156	00178	00219	
Cerebrum	00175	00174	00178	00195	00201		00245	
Cerebrum	00181	00180	00185	00192	00202	00224	00241	
Liver	00075	00089	00099	00112	00117	00132	00156	00282
Liver	00081	00094	00097	00121	00165	00187	00236	
After ½ hour	00082							
Voluntary muscle	00486	00472	00484	00499	00498			00617*
Voluntary muscle	00194							00210*

* Following day.

We found that the conductivity of all tissues remained practically unchanged during the first hour after removal from the body; i.e., provided the section had been kept at a constant temperature in a humid atmosphere. The earliest post-mortem changes in conductivity were found in the liver and the brain; the latest in voluntary muscle. No significant change was noted in the brain in less than one hour after removal. In two instances liver changes began in approximately one-half hour after the death of the animal (table 4). The observation of these changes led to the adoption of an unvarying routine, in which the liver was always the first tissue to be removed, sectioned and measured.

Digitized by Google

As a corollary to the measurements made specifically for the purpose of determining the onset of post-mortem changes it may be pertinent to note here that observations of the electric conductivity of the liver after it had been tied off for four hours, but left in situ, to produce an effectual hepatectomy, showed the conductivity was increased far above that observed in any experiment in which immediate measurements of the conductivity of the liver were made.

Post-mortem change was not the only factor to be considered, however. It is obvious that animal tissues present no such ideally uniform material as is ordinarily subjected to conductivity measurements. Even if it were possible to secure sections of exact uniformity throughout, completely filling the space between the electrodes, an ideal that thus far has not been satisfactorily attained, it would still be necessary to consider variations in the structure of different parts of the same organ, as well as variations between individual animals.

It was obvious at once that at best we could expect only to establish the limits of variations for each tissue, and that if the results of these measurements were to be of any general physiological value, they must represent the findings in a large number of individuals.

For all these reasons it seemed inadvisable to employ any technique which would delay the prompt measurement of sections after their removal from the animal; or would prevent the examination of a large number of animals, even though such a technique might markedly increase the precision of any single measurement. The precision of a single measurement is of little significance if it is obtained after such a lapse of time as to permit a change from the conditions in the living animal. Also if the normal variation in the conductivity of the same tissue in different animals is several per cent, as one would expect to be the case, it becomes important to multiply the *number of normal measurements* as well as to try for a high degree of precision in single measurements.

On the other hand the absolute maintenance of constancy of method is imperative and no effort has been spared to insure this requisite. Uniformity of technique in the choice of animals, the method of killing, the nature of treatment, the lapse of time and conditions under which the tissue was kept between the killing of the animal and the actual measurement, in the method of measurement and in all other factors has been maintained just as far as possible.

Animals which showed any abnormal condition either before or during treatment or at autopsy were always rejected. The weight

Digitized by Google

and temperature of each animal were recorded. The animals received their last feeding the evening before they were used, as the measurements were always made during the morning; calculations, testing and adjusting of apparatus and so forth being done during the afternoon. The animals were killed by stunning with a blow on the head and the immediate severance of the veins and arteries.

The liver was at once removed, sectioned, packed in warm tubes and placed in the saturated atmosphere of the constant temperature bath for immediate measurement.

In the earlier experiments the block of liver tissue was removed and kept as nearly intact as possible. As it proved difficult to secure sufficient uniformity of filling of the tubes by this method, in the later work the liver substance was separated from the connective tissue and the resultant soft mass was carefully transferred to the tubes. Histologic examination shows that this treatment separates the lobules from the connective tissue, but as the size of the individual liver cell is 5 to 8 microns there is no reason to believe that more than a very small percentage of the liver cells themselves has been destroyed.

The other tissues were removed and sectioned in turn—always in the same order—and measured immediately. The time between the removal of a tissue until its measurement seldom exceeded 20 minutes and under no circumstances was the section allowed to cool perceptibly.

By careful training and close coöperation between principals and assistants, the technique was developed to such a point that it was possible to prepare and measure two sections each from the liver, cerebrum, cerebellum, spinal cord, voluntary muscle and involuntary muscle (heart), and usually one section only from the adrenals and from the thyroid, within 45 minutes after the death of the animal. In the later work, when in the majority of animals measurements were made of only the brain and the liver, it was possible to make measurements of a number of animals each day, and thus secure nearly uniform conditions in the different animals.

At the beginning of the research as many sections as possible were made of each type of tissue until a minimum percentage of variation for each was established.

Thereafter, whenever the measurements of different sections of the same organ failed to agree, the higher value was in general the one accepted, for the reason that all sources of error under the conditions employed in this work were such as would diminish the conductivity. Thus if the tubes were incompletely filled, that is, if they contained

Digitized by Google

air spaces; if there was imperfect contact with the electrodes; if the material protruded beyond the tubes used in the earlier work, so that the length of the section was greater than that of the containing tube; if too great pressure was exerted; if the temperature of the section was reduced below the normal—any of these conditions would produce a measurement below the true conductivity value.

Two readings of each section were made, an interval of one minute being allowed between the successive readings. The magnitude of the current used was varied according to the resistance of the individual section. The relation of the area of the cross section to the length of the section of each type of tissue was kept within the range that experience showed would give the most favorable minima on the telephone.

Range of electrical conductivity of normal rabbit tissues. The accompanying table (table 5) gives the average measurements of the electrical conductivity of tissues from 91 normal rabbits. In addition to these measurements preliminary studies made during the development of the technique, bring the total number of normal rabbits studied to over one hundred.

Besides the tissues included in the table, measurements have been made of the thyroid, the adrenals, the kidneys and the spleen. The variation in these measurements was so great that no averages for these tissues have been made and it remains to discover some method by which accurate measurements of these tissues and increased accuracy in the measurement of the spinal cord, of the heart and of voluntary muscle may be secured.

It will be noted that with the exception of the spinal cord, the order of magnitude of the conductivity values of the tissues included in table 6 never varied.

It was the initial plan to establish a normal range of the conductivity of the various tissues to be used as the basis of comparison for the tissues of all subsequently treated animals. Accident, however, showed the futility of this plan.

During the period in which groups I to III were measured, (November 1918 to February 1919) the animals had been kept in airy, cool quarters in the country, and provided with an open air run. In April they were removed to a typical animal room which, although well lighted and ventilated, was a great contrast to the former quarters. The effect upon the animals which were transferred is indicated by the measurements of group IV. The measurements of this group as well as of all

Digitized by Google

TABLE 5

Measurements of the electrical conductivity of normal rabbit tissues

Group		Number of animals	Cerebrum	Average deviation per cent	Number of animals	Cerebellum	Average deviation per cent	Number of animals	Liver	Average deviation per cent
I.	11/14–11/22/18	7	00186	1.2	7	00155	2.6	9	00075	4.0
II.	12/14–12/21/18	6	00189	1.4	6	00164	0.8	3	00072	5.9
III.	2/12– 2/19/19	5	00192	1.6	6	00162	5.3	5	00074	2.2
IV.	*4/21– 5/ 6/19*	*6*	*00138*	2.0	*5*	*00117*	*4.0*	*5*	*00086*	*5.0*
V.	5/12– 5/27/19	6	00185	1.5	7	00137	3.0	4	00071	3.0
VI.	6/11– 6/18/19	8	00172	1.7	8	00131	1.0	6	00101	1.7
VII.	10/1 –10/14/19	13	00177	1.5	11	00134	2.0	11	00097	3.7
VIII.	10/17–10/27/19	6	00168	1.5	6	00134	3.4	6	00094	4.0
IX.	11/1 –11/15/19	9	00181	1.3	9	00135	2.2	8	00096	5.0
X.	11/22–12/ 2/19	4	00179	1.4	5	00128	2.4	5	00061	5.7
XI.	12/3/19–1/6/20	7	00172	2.4	8	00127	2.8	8	00091	9.0
XII.	1/6 – 1/26/20	5	00161	4.5	5	00129	3.2	4	00077	6.5
XIII.	2/3 – 2/28/20	8	00179	1.9	8	00126	2.3	7	00063	6.3

Group	Number of animals	Spinal fluid	Average deviation per cent	Number of animals	Blood	Average deviation per cent	Number of animals	Bile	Average deviation per cent	Number of animals	Heart	Average deviation per cent	Number of animals	Voluntary muscle	Average deviation per cent	Number of animals	Lung	Average deviation per cent	Number of animals	Spinal cord	Average deviation per cent
I.													8	00591	9.5				7	00146	5.2
II.													4	00745	5.0				6	00180	5.9
III.													4	00655	2.6				4	00116	6.0
IV.													*6*	*00345*	*7.4*				*7*	*00259*	*3.8*
V.										5	00117	5.4	4	00545	4.9						
VI.																					
VII.	4	0194	0.6	5	00852	2.0	4	0152	0.6												
VIII.	4	0182	3.4	5	00774	2.5	4	0158	2.0												
IX.	6	0162	2.0	7	00739	1.3	8	0144	1.8												
X.	3	0184	3.0	4	00772	2.8	4	0139	5.5												
XI.	5	0187	5.8	7	00746	2.0	7	0164	5.0												
XII.	5	0166	2.4	4	00737	3.2	4	0140	2.6												
XIII.				4	00847	2.2	5	0159	3.9	8	00105	5.1	3	00666	1.6	7	000565 (Range 000507 to 000709)	5.3	5	00167	7.2

Digitized by Google

treated animals among those that were transferred from the country quarters have been discarded since the great discrepancy between these measurements and those in the earlier as well as in the later groups illustrates most strikingly the effect of variations in environment, season, etc. In this instance the effect of moving and of the changed environment was sufficient to put the animals in the abnormal class, although at autopsy no sign of disease could be discovered. We then began securing animals from a dealer in the country in small groups so that the measurements of all treated animals could be compared with the measurements of normal animals of the same group.

No conclusions have been drawn from findings in any series unless the difference between the electrical conductivity of the organ in the

TABLE 6

Range of electrical conductivity of various normal rabbit tissues arranged in the order of magnitude of their conductivity values

Tissue	Conductivity
Spinal fluid	0.0164–0.0194
Bile	0.0139–0.0164
Blood	0.00739–0.00852
Voluntary muscle	0.00580–0.00745
Cerebrum	0.00161–0.00198
Cerebellum	0.00126–0.00151
*Heart	0.00105–0.00117
Liver	0.00061–0.00101
Lung	0.00051–0.00071

* On account of the wide range of the individual measurements of the heart muscle and the fact that in our earliest series its conductivity appeared to be higher, its place in this table may be questioned.

treated animal and the average measurement of the normal organ in the same group has been greater than the established average deviation of the normal measurements in that group.

It will be noted that throughout these researches, there has been no exception to what appears to be the normal relationship between the cerebrum and the cerebellum in the adult animal; i.e., in every adult animal the conductivity of the cerebrum has been greater than that of the cerebellum. This constant relationship was observed also by Doctor Obear in his preliminary studies. In order to discover whether or not this relation is a characteristic of adult life only, series of fetuses and of young rabbits were measured, and the significant observation was made that in fetuses and immediately after birth *the conductivity of the cerebellum was higher than that of the cerebrum.* In

Digitized by Google

TABLE 7

Relation between the electrical conductivity of the cerebrum and of the cerebellum in fetuses and in young rabbits

	CEREBRUM	CEREBELLUM	LIVER
Mother I	*00163*	*00125*	*00094*
Fetus 1	00087	00125	00049
2	00112	00137	00046
3	00103	00113	
4	00110	00119	00052
5	00070	00136	00048
Mother II	*00185*	*00138*	*00090*
Fetus 1	00097	00099	00033
2	00107		00033
3	00097	00099	
4	00096	00116	00057
Mother III (nearly at term)	*00143*	*00114*	*00099*
Fetus 1	00109	00115	00048
2	00086	00125	00059
3	00104	00119	00037
4	00076	00116	00058
5	00096	00108	00039
6	00092	00094	00045
Approximately 1-2 hours old			
1	00103	00120	00081
2	00096	00131	00088
24 hours old			
1	00122	00114	00072
2	00130	00116	00102
32 hours old			
1	00135	00118	00046
2	00107	00128	00059
4 days old			
1	00111	00140	00092
2	00132	00114	00072
3	00127	00094	00076
6 days old			
1	00145	00140	00078
2	00183		00075

Digitized by Google

TABLE 7—*Concluded*

	CEREBRUM	CEREBELLUM	LIVER
7 days old			
1	00165	00149	00068
2	00156	00162	00066
10 days old			
1	00159	00157	00102
2	00176	00132	00061
1 month, 20 days			
1	00171	00122	00084
2	00172	00120	00062
2–3 months			
1	00188	00158	00118
2	00195	00147	00099

most of the fetuses measured the conductivity of the cerebellum was as high as in the average adult, while the conductivity of the cerebrum in the fetus and in the newborn rabbits was far below normal. The rise of the conductivity of the cerebrum to the normal level apparently coincides with the emerging of the young rabbit from the nest and the inauguration of its conscious life as an independent individual (table 7, chart 1). As will be noted in the chart and table, the conductivity of the liver of the fetus is far below that of the normal adult, but apparently rises to the normal level at birth. It should be noted that on account of the very small size of the fetal cerebellum, it was necessary to use a special electrode and minute glass tubes, so that the possibility of error in the measurements of the cerebellum was greater than in the case of the cerebrum.

An interesting corollary to these observations in rabbits may be noted here. Permission was granted for securing sections of the brains of two patients who died in the hospital on the same day. One died from carcinoma of the stomach and had been conscious until death; the other had been unconscious for days before his death, which was caused by a brain tumor.

As is shown by table 8, in the patient conscious until death, the conductivities of the cerebrum and the cerebellum while low, undoubtedly as the result of the exhaustion of prolonged disease, nevertheless preserved the relationship observed in all our adult animals; viz., the

Digitized by Google

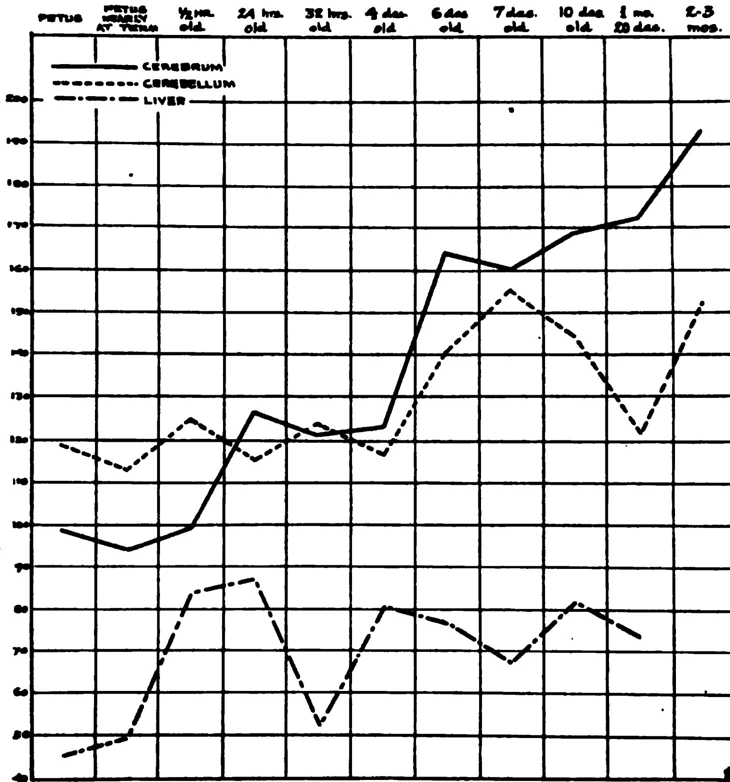


Chart 1. Progressive changes in the electrical conductivity of the brain and the liver of rabbits from just before birth to the age of 3 months.

TABLE 8

Comparison between the relative conductivities of the cerebrum and of the cerebellum of two patients—one conscious until death and one unconscious for days before death

	CEREBRUM	CEREBELLUM
I. Patient conscious until death		
Section I	00143	00116
Section II	00120	00116
Section III	00136	00107
II. Patient unconscious for days before death		
Section I	00139	00181
Section II	00135	00157
Section III	00134	00175

Digitized by Google

conductivity of the cerebrum was higher than that of the cerebellum. In the patient who had been unconscious, this relationship was reversed, the conductivity of the cerebellum being higher than that of the cerebrum. It will be noted also that in this case the value of the conductivity of the cerebellum is very high.

TABLE 9

Relation between the electrical conductivity of the gray matter and of the white matter of the cerebrum

SECTION	GRAY MATTER	WHITE MATTER
1	00213	00117
2	00218	00106
3	00209	00115
4	00227	00147
5	00215	00133
6	00271	00164
7	00202	00139
8	00232	00115
9	00260	00150
10	00240	00143

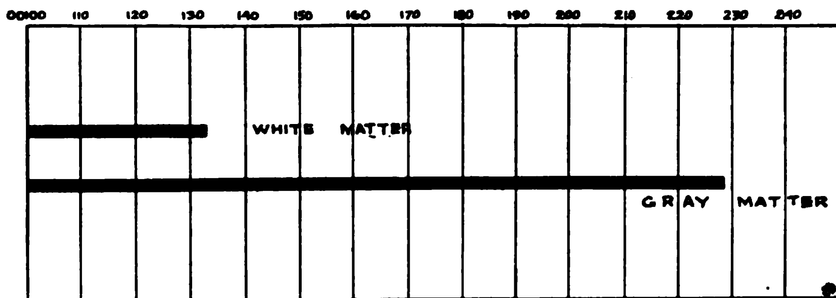


Chart 2. Comparison of the electrical conductivity of the gray and of the white matter of the cerebrum. (Actual measurements.)

A number of measurements has been made to determine whether or not there is a regional variation in the conductivity of the cerebrum. While these preliminary studies are suggestive, the only result which seems sufficiently established to be noted here is the relation between the conductivities of the gray and of the white matter. As shown by table 9 and chart 2, in every measurement thus far made, the conductivity of the gray matter has been markedly higher than that of the white matter.

Digitized by Google

EFFECTS OF EXHAUSTION DUE TO VARYING CAUSES UPON THE ELECTRICAL CONDUCTIVITY OF THE BRAIN AND THE LIVER. In selecting the types of exhaustion to be included in this research, we were guided by previous researches in order that we might discover whether or not any measurable relation could be established between the histological changes observed in exhaustion, in particular those in the brain, and the electrical conductivity. That this correlation might be fairly made, we have been constantly guided by the data of the previous researches in the treatment of the animals, dosage, protraction of stimulation, etc.

I. Insomnia. Thirteen Belgian hares of weights varying from 1.414 to 2.588 kgm. were kept awake continuously for 96 hours. During

TABLE 10

Effect of prolonged insomnia—96 hours—upon the weight and temperature of rabbits

RABBIT	INITIAL WEIGHT	FINAL WEIGHT	INITIAL	FINAL
	gm.	*gm.*	*temp.*	*temp.*
I	2.588	2.595	39.6	40.2
II	2.206	1.605	39.0	38.8
III	2.283	2.400	39.6	40.0
IV	2.243	2.254	39.0	39.0
V	2.158	2.215	39.6	39.6
VI	2.343	2.421	39.6	39.6
VII	2.223	2.321	39.6	39.2
VIII	1.730	1.715	39.0	40.0
IX	1.828	2.003	39.2	39.0
X	1.716	1.778	39.0	39.0
XI	1.793	1.630	39.2	39.0
XII	1.414	1.473	38.8	41.0
XIII	2.348	2.328	39.8	39.6

this time they were confined in a large airy room and were given abundant food and water. The animals were kept awake by constant but gentle prodding; they were not hurt in any way, nor at any time did they manifest any discomfort beyond their attempts to settle into corners where they might be left alone. Most of the animals ate and drank freely throughout the insomnia period. Table 10 gives the weight and temperature of each at the beginning and at the end of the period of insomnia.

At the end of the insomnia period four of the rabbits were killed at once and conductivity measurements made. Four were put into a darkened room and left undisturbed for 6 hours, when they in turn

Digitized by Google

TABLE 11

Effect of insomnia and of insomnia followed by periods of rest on the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUP	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
12/14–12/21/18	*Normal II*	*6*	*00189*	*1.4*		*6*	*00164*	*0.8*		*6*	*00072*	*5.9*	
a	Insomnia	4	00184	1.2	−2.6	4	00154	4.8	−6.09	5	00075	5.8	+4.1
b	Insomnia + 6 hours rest	5	00186	1.5	−1.5	5	00140	3.1	−14.6	5	00077	3.6	+6.9
c	Insomnia + 1–2 weeks rest	4	00203	4.0	+7.4	4	00155	3.0	−5.4	4	00089	3.9	+23.6

Digitized by Google

were killed and conductivity measurements made. The remaining four were kept undisturbed for from 7 to 14 days.

The average conductivities of the cerebrum, cerebellum and liver in each of these groups are shown in table 11 and chart 3.

II. Fright and exertion. Each of six rabbits was frightened until exhausted by a dog which kept them in a state of intense nervous excitement by barking and thwarted attacks until they were prostrated

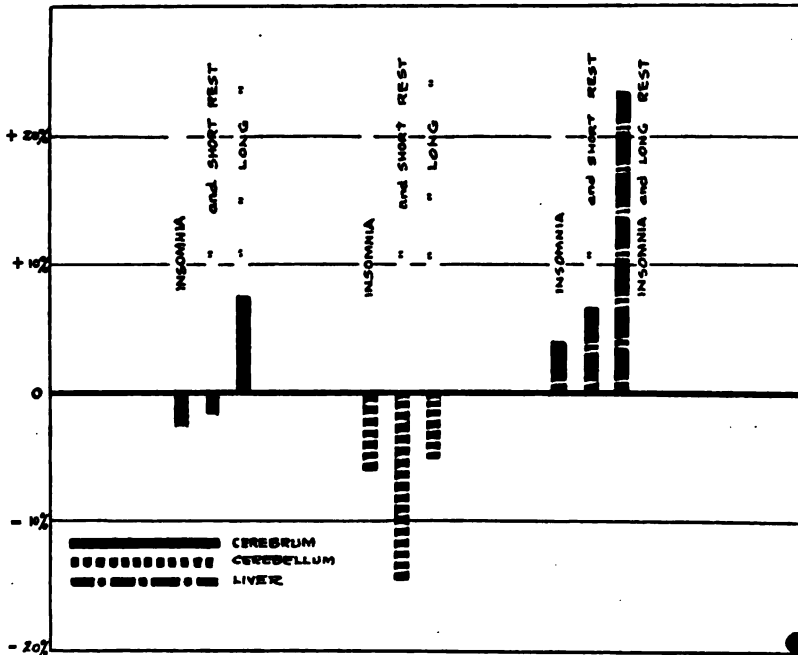


Chart 3. Comparison of the immediate effect of prolonged insomnia on the electrical conductivity of the brain and of the liver with the effect of short and of prolonged periods of rest. (Percentile variations from normal.)

by the resultant exhaustion. The effect upon the conductivity of the brain and the liver is shown in table 12 (a).

III. Adrenalin—repeated doses. Repeated doses—2 to 3—of 1-1000 adrenalin (P. D. & Co.) were given to each of 8 rabbits at intervals of from 10 to 20 minutes according to the degree of reaction. The average dose—intravenous—was 0.4 cc. per kgm.; to two rabbits twice this dose was given intramuscularly. Typical changes in pulse and respiration were produced in each animal with ultimate prostration. The conductivity changes are shown in table 12 (e).

Digitized by Google

TABLE 12

Effects of exhaustion from various causes upon the electrical conductivity of the liver

DATE OF EXPERIMENT	STIMULUS		NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
						per cent				*per cent*				*per cent*
12/14–12/21/18	*Normal II*		*6*	*00189*	*1.4*		*6*	*00164*	*0.8*		*6*	*00072*	*5.9*	
12/5–12/14/18	Fright	(a)	6	00180	3	− 4.7	6	00152	1.3	− 7.3	6	00118	8	+63.8
11/26–12/10/18	Insomnia	(b)	4	00184	1.2	− 2.6	4	00154	4.8	−6.09	4	00075	5.8	+ 4.1
1/3–2/19/19	*Normal III*		*5*	*00192*	*1.6*		*5*	*00162*	*5.3*		*5*	*00074*	*2.2*	
1/8–1/10/19	Surgical shock	(c)	5	00168	2.4	−12.5	5	00154	1.7	−4.9	6	00091	5.5	+22.9
12/8–12/18/19	Diphtheria toxin	(d)	5	00179	2.7	− 6.7	6	00156	3.1	−3.7	4	00069	3	− 6.7
1/3–1/7/19	Adrenalin injection	(e)	8	00184	2	− 4.1	7	00154	1.4	−4.9	7	00094	3.4	+27.02
1/27–2/17/19	Thyroid feeding	(f)	5	00175	2.5	− 8.8	5	00144	3.6	−11.1	5	00074	4.4	0
1/31–2/8/19	Hydrochloric acid	(g)	9	00168	2.7	−12.5	9	00144	2.7	−11.1	9	00093	4	+25.6
1/30–2/13/19	Ether—4 hours continuous	(h)	6	00177	1.6	− 7.8	6	00130	2.6	−19.7	6	00097	5.7	+31.08
2/13–2/19/19	Nitrous oxid—4 hours continuous	(i)	6	00184	3.4	− 4.1	5	00139	2.3	−14.1	6	00094	7	+27.02
5/12–5/27/19	*Normal V*		*6*	*00185*	*1.5*		*7*	*00137*	*3*		*4*	*00071*	*3*	
5/14–5/26/19	Strychnin	(j)	5	00146	3.9	−21.08	4	00130	5.4	−5.1	5	00092	3.3	+29.5

Digitized by Google

IV. Surgical shock. Each of six rabbits was subjected, under ether, to severe trauma of the intestines and abdominal walls for periods of from 30 to 45 minutes. The resultant conductivity changes are shown in table 12 (c).

V. Prolonged ether and prolonged nitrous oxid anesthesia. Six rabbits were subjected to 4 hours' continuous ether anesthesia; and six to continuous nitrous oxid anesthesia of the same duration with resultant conductivity changes which are shown in table 12 (h) and (i).

VI. Thyroid feeding. Six rabbits in fine general condition and of approximately equal weight were each given 5 grains of thyroid extract daily for 3 weeks. All but one showed at first a loss of appetite, with a later increased appetite but a continued loss of weight. With the exception of the one referred to above, which seemed to thrive, the fur of all became rough and coarse in appearance and was shed abundantly; they became nervous and excitable; the eyes were staring in appearance; the skin felt hot to the touch although the clinical thermometer showed no change in temperature. In brief, the animals manifested the typical signs of thyroid intoxication. The probable reason for the exception of the one rabbit noted above appeared at autopsy, which showed a minute and pale thyroid gland as compared with enlarged vascular glands in each of the others.

The losses of weight in each animal were as follows: 18 per cent, 29 per cent, 15 per cent, 36.6 per cent, with a loss of but 4.5 per cent in the exceptional one. One animal died after 2 weeks, with a loss in weight of 33.8 per cent. At the termination of 3 weeks the animals were killed and conductivity measurements made (table 12 (f)). It should be noted that in this group the thyroid feeding was protracted until the stage of exhaustion had been reached. Earlier effects are described in a later section.

VII. Hydrochloric acid. In each of four rabbits 1 cc. of hydrochloric acid—10 per cent—was injected in the femoral vein. Each showed an immediate reaction registered in circulatory and respiratory changes, convulsive movements and prostration. The animals were killed in from four to twelve minutes after the injection and conductivity measurements made (table 12 (g)).

VIII. Strychnin. On account of the wide variation in the response of individual rabbits to strychnin, in this series in which it was desired to produce a massive effect the dosage varied from the just tetanic (0.155 mgm. per kgm. intravenous, Sollman) to the just fatal (0.36 mgm. per kgm. intravenous, Sollman), the dose being repeated if required to

Digitized by Google

produce sufficient reaction. Five animals were included in the series. In each a reaction varying from a general tremor to severe convulsions was produced.

While on account of the variation in dosage and clinical results this is not considered a satisfactory series, the contrast in the conductivity findings to those in a subsequent series in which the incipient effects were noted, is so marked that the series has been included. (Table 12, (j)).

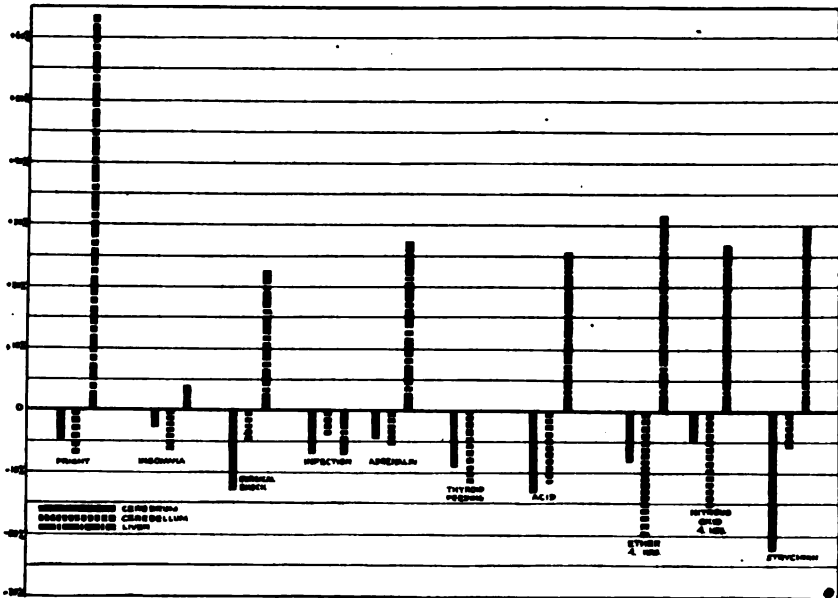


Chart 4. Effects of exhaustion due to varying causes upon the electrical conductivity of the brain and liver. (Percentile variations from the normal.) Note that in only one instance—infection—is the conductivity of the liver decreased: in thyroid feeding the average conductivity of the liver was unchanged.

Summary. A study of table 12 and chart 4 shows that in each type of exhaustion there included the conductivity of the cerebrum and of the cerebellum was diminished and the conductivity of the liver was increased except in exhaustion due to thyroid feeding in which case the average conductivity of the liver was unchanged from the normal. Chart 5 shows that with the exception of exhaustion produced by adrenalin injection and by prolonged nitrous oxid anesthesia, in every instance the average conductivity of the cerebrum in exhaustion fell

Digitized by Google

beyond the lowest individual normal measurement included in the normal group with which comparison is made. If one allows for the average deviation of the cerebellum and of the liver in both the exhausted and the normal animal, in certain instances the apparent change in exhaustion falls within those limits, nevertheless the marked downward tendency in the cerebrum and the cerebellum, and the upward tendency in the liver are obvious, as is shown also in chart 5 in which are charted the actual average deviations from the normal for all measurements in which normal group III was the basis for comparison.

INCIPIENT EFFECTS OF EXHAUSTION-PRODUCING AGENTS ON THE ELECTRICAL CONDUCTIVITY OF THE BRAIN AND THE LIVER. Previous researches and clinical observations had indicated the presence of an incipient stage of shock marked by hyperchromatism of the brain cells

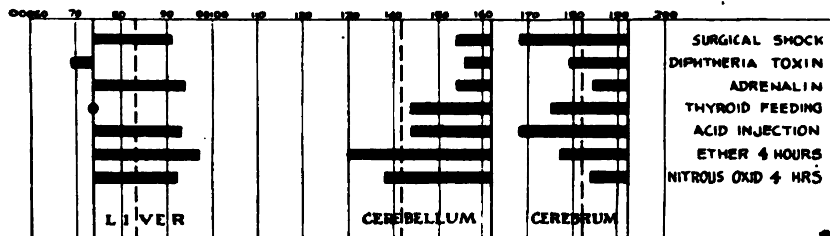


Chart 5. Changes in the electrical conductivity of the brain and of the liver in exhaustion from various causes. (Actual deviations from the normal.) Note the vertical dotted lines which indicate the average deviation of each tissue from the normal average indicated by the solid vertical lines.

as compared with hypochromatism after shock had become established. To determine whether or not a corresponding early increase in the electric conductivity of the brain precedes the ultimate decreased conductivity after the state of exhaustion or shock is established, a number of studies of the incipient effects of some of the shock-producing agents noted above was made.

I. Incipient effects of intense trauma. Under light ether anesthesia two groups of rabbits were subjected to extreme shock-producing manipulations for periods of 1 and of 5 minutes respectively. They were killed immediately and electric conductivity measurements made (table 13 (a), chart 6).

Incipient effects of strychnin. Each of five rabbits was given a fatal dose (0.36 mgm. per kgm.) intravenously and killed 1 minute after the injection (table 13 (d) chart 6).

Digitized by Google

TABLE 13

Incipient effects of various exhaustion producing agents upon the electrical conductivity of the brain and the liver

NUMBER AND DATE OF NORMAL GROUP FOR COMPARISON	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
6/11–6/18/19	*Normal VI*	8	*00172*	*1.7*		8	*00131*	*1*		6	*00101*	*1.7*	
6/ 9/19	(a) Surgical shock—1 min.	5	00189	1.0	+ 9.8	6	00138	1.3	+5.3	4	00108	1.9	+ 6.9
6/ 6/19	5 min.	3	00175	0.8	+ 1.7	3	00142	1.9	+8.3	3	00116	6.5	+14.8
6/10/19	(b) Ether—stage of excitement	3	00195	0.6	+13.3	3	00126	2.3	−3.8	2	00119	4.2	+17.8
6/11/19	(c) Nitrous oxid—stage of excitement	3	00174	1.6	+ 1.1	3	00139	6.3	+6.1	3	00099	6.6	− 1.9
6/12–6/16/19	(d) Strychnin	4	00193	3.0	+12.2	4	00143	2.1	+9.1	3	00092	7.3	− 8.9
6/13–6/16/19	(e) Adrenalin	4	00181	3.4	+ 5.2	4	00129	2.7	−1.5	4	00095	4.6	− 5.9

Digitized by Google

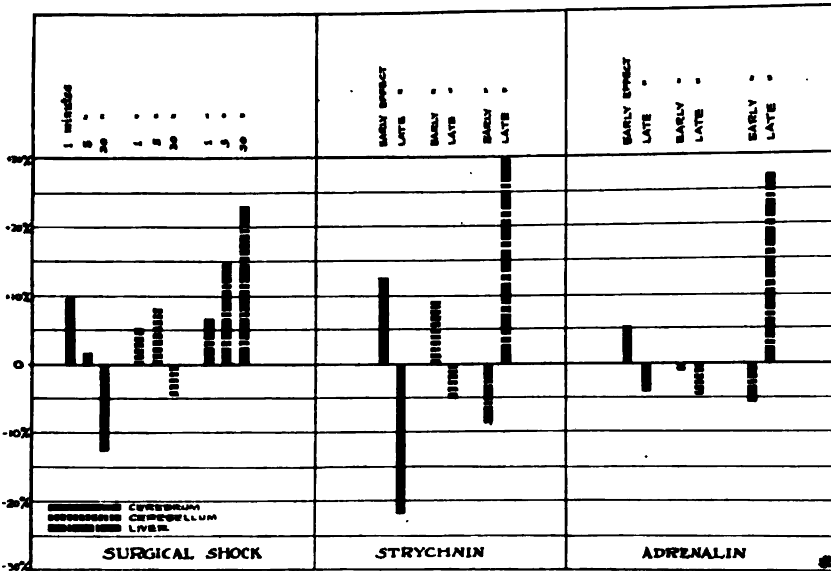


Chart 6. Comparison of the incipient and late effects of stimulation by various agents on the electrical conductivity of the brain and of the liver. (Percentile variations from the normal.)

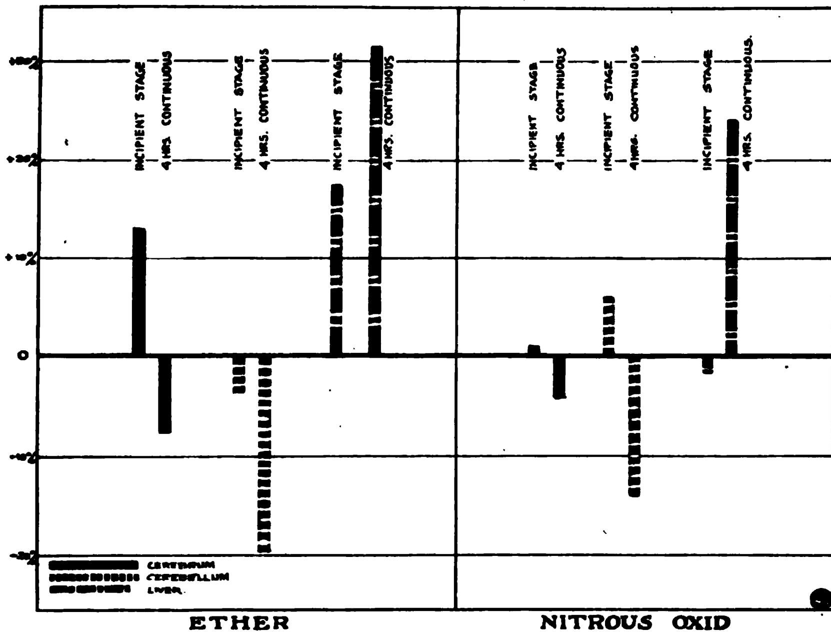


Chart 7. Incipient and late effects of ether and of nitrous oxid-oxygen anesthesia on the electrical conductivity of the brain and the liver. (Percentile variations from the normal.)

Digitized by Google

Incipient effects of adrenalin. Each of three rabbits was given an intravenous injection of 0.4 cc. per kg. of 1-1000 adrenalin (P. D. & Co.) and killed 1 minute later (table 13 (e) chart 6).

Incipient effects of ether and of nitrous oxid anesthesia. To one of two groups of rabbits ether was administered for from 2 to 5 minutes; nitrous oxid being administered to the other group for like periods. Each animal was killed just at the termination of the first stage of anesthesia—the stage of excitement (table 13 (b and c) chart 7).

Diphtheria toxin. To various groups of rabbits twice the lethal dose of diphtheria toxin (P. D. & Co.) was given intravenously, the

TABLE 14

Progressive effects of the injection of diphtheria toxin upon the electrical conductivity of the brain

DATE OF EXPERIMENT	PERIOD AFTER INJECTION OF TOXIN	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*
5/12–5/27/19	*Normal V*	*6*	*00185*	*1.5*		*7*	*00137*	*3*	
6/2–6/4/19	15 min.	2	00226	1.9	+22.0	3	00137	0.9	0
6/2–6/4/19	30 min.	3	00194	1.3	+ 4.8	3	00128	1.8	− 6.5
5/27–5/29/19	1 hour	3	00180	1.7	− 2.7	3	00109	0.5	−20.4
12/3/19–1/6/20	*Normal XI*	*7*	*00172*	*2.4*		*8*	*00127*	*2.8*	
12/15/19–1/2/20	15 min.	3	00189	1.2	+ 9.8	3	00154	1.7	+21.2
1/7/20	30 min.	2	00174	3.8	+ 4	2	00131	0	+ 3.1
1/3–2/19/19	*Normal III*	*5*	*00192*	*1.6*		*5*	*00162*	*5.3*	
2/8–2/18/19	4 hours	5	00179	2.7	− 6.7	6	00156	3.1	− 3.8

animals being killed at intervals varying from 5 minutes to 1 hour after the dose was received—the progressive effects are shown in table 14 and in chart 8.

SUMMARY. With every exhaustion-producing agent studied, the initial effect was an increased conductivity of the cerebrum followed by a decrease to below the normal when the stage of exhaustion was reached. The early effect of stimulation upon the cerebellum appeared to vary, but a study of the clinical behavior of the animals, especially of the initiation of the respiratory and circulatory changes, together with an examination of the individual measurements, would

Digitized by Google

seem to indicate that a like unvarying rule exists in the case of the cerebellum, but that the protraction of the incipient stage is shorter than in the case of the cerebrum. Many additional experiments are required to establish this point.

A study of the individual measurements of the liver would appear to indicate an immediate tendency to decrease followed by an increase to above the normal.

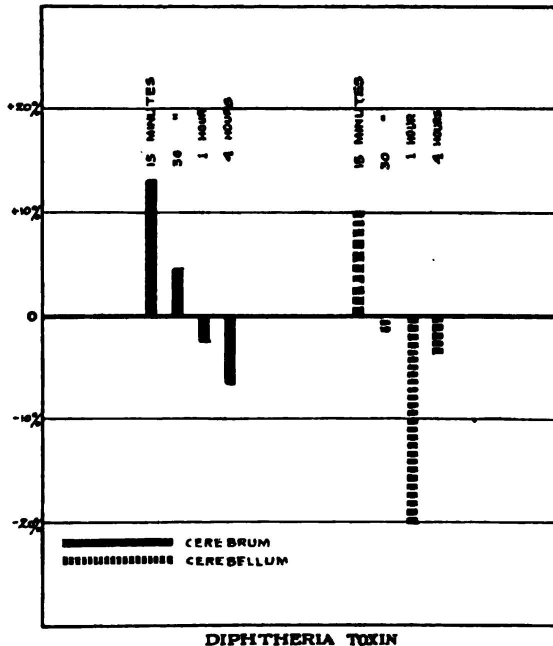


Chart 8. The incipient and later effects of the injection of diphtheria toxin on the electrical conductivity of the brain. (Percentile variations from the normal.)

THE EFFECTS OF DIPHTHERIA TOXIN IN THE PRESENCE OF MORPHIN ON THE ELECTRICAL CONDUCTIVITY OF THE BRAIN AND LIVER. The control of infection by morphin, so strikingly illustrated by the Alonzo Clark treatment of peritonitis, and the comparison of the histologic effects of diphtheria toxin alone and in the presence of morphin, led us to perform a series of experiments to determine whether or not the protective effect of morphin would be manifested by any diminution of the conductivity changes produced by diphtheria toxin alone.

Digitized by Google

TABLE 15

Comparison of the effects of diphtheria toxin alone and of diphtheria toxin in the presence of morphin on the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUP	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
12/14–12/21/18	*Normal III*	*6*	*00192*	*1.6*		*6*	*00162*	*5.3*		*3*	*00074*	*2.2*	
2/8 –12/18/19	Diphtheria toxin	5	00179	2.7	−6.7	6	00156	3.1	−3.7	4	00069	3.0	− 6.7
2/6 – 2/13/19	Morphin	6	00185	4.6	−3.6	6	00148	4.1	−8.6	6	00065	6.9	−12.2
2/6 – 2/18/19	Diphtheria toxin and morphin	6	00187	2.6	−2.6	6	00164	3.2	+1.2	6	00075	4.2	+ 1.4

Digitized by Google

Coincidently with the series of experiments described above in which the rabbits were killed 4 hours after the intravenous injection of twice the lethal dose of diphtheria toxin, to each of another group 5 grains of morphin were given hypodermically in two doses, 1 hour apart; twice the lethal dose of diphtheria toxin being given intravenously

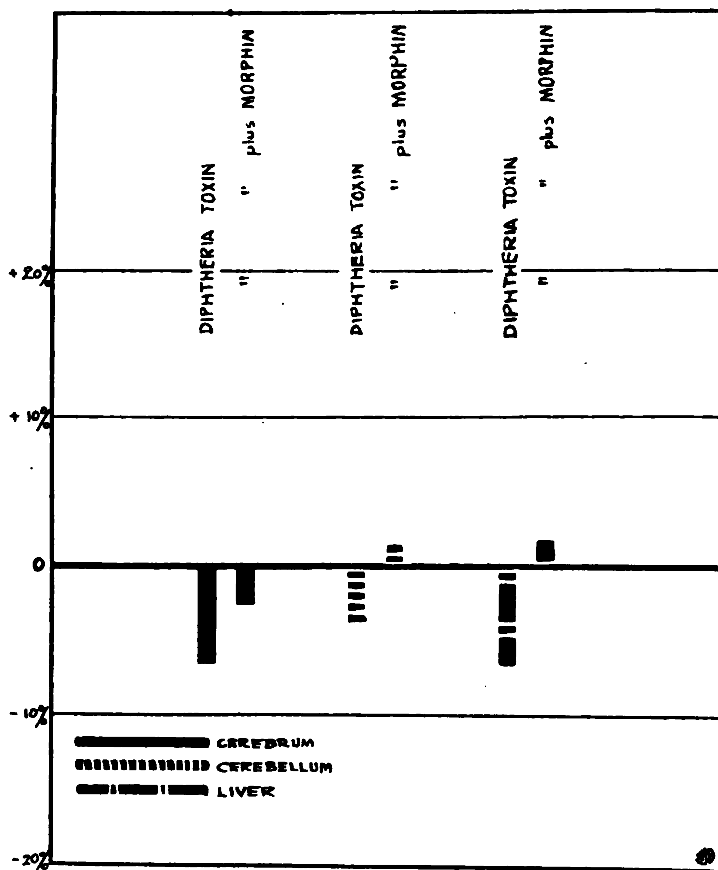


Chart 9. Comparative effects of the injection of diphtheria toxin alone and in the presence of morphin on the electrical conductivity of the brain and the liver. (Percentile variations from the normal.)

15 minutes after the second dose of morphin was received. The animals were killed 4 hours after receiving the diphtheria toxin.

To each of a third group morphin alone was administered as above, and the animals killed 4 hours after the second dose. The conductivity measurements are given in table 15, and illustrated in chart 9.

Digitized by Google

Effects of thyroid feeding, of iodin and of adrenalin in the presence of each, upon the electric conductivity of the brain and the liver. In a preceding section we have shown that the late effects of thyroid feeding are identical with the late effects of other exhaustion-producing agents.

Three later series of experiments were performed to discover the earlier effects of thyroid feeding and what if any, effect upon the conductivity of the brain is produced by adrenalin in the presence of thyroidism, or of iodism.

The results of these series are shown in tables 16 and 17 and in charts 10 and 11, in which have been included also for ready comparison the effects of thyroid feeding to the point of exhaustion, and the immediate effect of the injection of adrenalin.

The animals included in table 16, series II and III, and chart 10 had been given 2 to 3 grains of thyroid extract daily for a period of 4 weeks. It will be noted that while thyroid extract alone increases the conductivity of the cerebrum and of the cerebellum, the injection of adrenalin in the thyroid-fed animals produced a tendency to return toward or below the normal.

In table 17 and chart 11 the same contrast in the effects of iodoform and of iodoform plus the injection of adrenalin upon the conductivity of the cerebrum and of the cerebellum will be noted; i.e., iodoform alone increases the conductivity of the brain, this effect tending to be neutralized by adrenalin.

Each of the animals in the iodoform series had received an intraperitoneal injection of 75 grams of iodoform introduced through a small abdominal opening. The incision was closed and the animals killed on the following day. Each showed febrile phenomena. There was an early rise of temperature of from 0.4 to 1.3°C. which was persistent in all but three of the cases; in those three the temperature dropped to from 0.1 to 1.0°C. below the initial temperature.

That the increased conductivity produced by iodoform cannot be due to the permeation of the tissues by the iodine is shown in series II, table 17, in which, while the conductivity of the cerebrum and of the cerebellum is markedly increased, the conductivity of the liver is practically unchanged.

An essential corollary to these experiments would be the measurement of the conductivity of the brain in thyroidectomized animals after the injection of iodoform.

Digitized by Google

TABLE 16

Early and late effects of thyroid feeding and thyroid feeding plus adrenalin upon the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
Series I													
1/3 – 2/19/19	*Normal III*	*5*	*00192*	*1.6*		*5*	*00162*	*5.3*		*5*	*00074*	*2.2*	
1/27– 2/17/19	Thyroid feeding, late effects	5	00175	2.5	– 8.8	5	00144	3.6	–11.1	5	00074	4.4	0
Series II													
12/3/19–1/6/20	*Normal XI*	*7*	*00172*	*2.4*		*8*	*00127*	*2.8*		*8*	*00091*	*9*	
12/3 –12/ 9/19	Thyroid feeding	3	00189	0.9	+ 9.8	3	00147	0.9	+15.7	4	00080	2.5	–12.08
12/3 –12/ 5/19	Thyroid feeding plus adrenalin	2	00188	0.3	+ 9.3	3	00127	1.1	0	2	00070	0	–23.07
Series III													
2/ 3– 2/28/20	*Normal XIII*	*8*	*00179*	*1.9*		*8*	*00126*	*2.3*		*7*	*00063*	*6.3*	
2/17– 2/18/20	Thyroid feeding	2	00199	2.6	+11.1	1	00137		+ 8.7	2	00061	4.1	– 3.1
2/17– 2/18/20	Thyroid feeding plus adrenalin	3	00163	2.1	– 8.9	3	00128	2.3	+ 1.6	3	00050	11.3	–20.6
Early effects of adrenalin alone													
6/11– 6/18/19	*Normal VI*	*8*	*00172*	*1.7*		*8*	*00131*	*1*		*6*	*00101*	*1.7*	
6/13– 6/16/19	Adrenalin	4	00181	3.4	–33.4	4	00129	2.7	– 1.5	4	00095	4.6	– 5.9

Digitized by Google

TABLE 17

Effect of iodoform and of iodoform plus adrenalin on the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL
					per cent				*per cent*				*per cent*
Series I.													
6/11–6/18/19	*Normal VI*	*8*	*00172*	*1.7*		*8*	*00131*	*1*		*6*	*00101*	*1.7*	
6/17–6/18/19	Iodoform	3	00193	0.5	+12.2	4	00134	1.1	+ 2.2	4	00131	7.3	+29.7
Series II.													
2/3–2/28/20	*Normal III*	*8*	*00179*	*1.9*		*8*	*00126*	*2.3*		*7*	*00063*	*6.3*	
2/6/20	Iodoform	2	00194	4	+ 8.3	2	00140	4	+11.1	2	00064	6.7	+ 1.5
2/5/20	Iodoform plus adrenalin	2	00176	2.2	− 1.6	2	00124	0	− 1.5	2	00083	1.2	+31.7
Early effects of adrenalin alone													
6/11–6/18/19	*Normal VI*	*8*	*00172*	*1.7*		*8*	*00131*	*1*		*6*	*00101*	*1.7*	
6/13–6/16/19	Adrenalin	4	00181	3.4	+ 5.2	4	00129	2.7	− 1.5	4	00095	4.6	− 5.9

Digitized by Google

Opposite effects of acid and of alkali injection upon the electrical conductivity of the brain and the liver. In each of 6 rabbits 10 cc. of a saturated solution of sodium bicarbonate were slowly injected through the marginal ear vein. Each animal was killed 2 hours after the injection and sections taken for conductivity measurements.

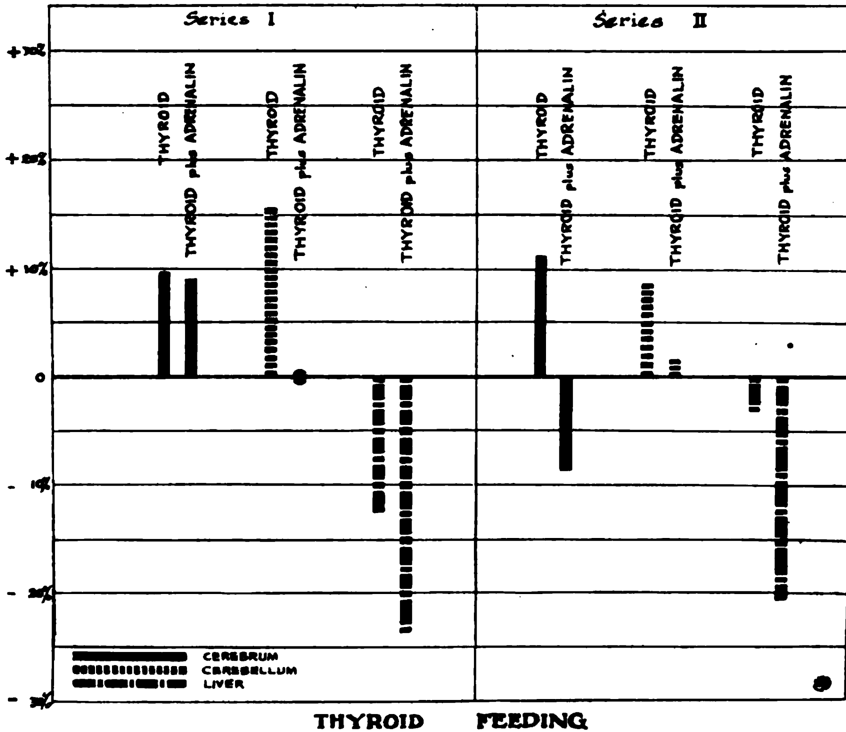


Chart 10. Changes in the electrical conductivity of the brain and of the liver produced by thyroid feeding and by thyroid feeding plus the injection of adrenalin. (Percentile variations from the normal.)

As will be seen in table 18 and chart 12, the injection of sodium bicarbonate increased the conductivity of the brain and decreased the conductivity of the liver, while the injection of hydrochloric acid, as described in a preceding section of this report, decreased the conductivity of the brain and increased the conductivity of the liver.

Miscellaneous group—showing the effects of various agents upon the electric conductivity of the brain. In this section are

Digitized by Google

included a number of preliminary studies. No comment is made, as they should be extended before any conclusions can be drawn. The indications are sufficiently shown in tables 19 and 20.[1]

I. Magnesium sulphate—calcium chloride. To each of four rabbits 6 cc. per kgm. of a 25 per cent solution of magnesium sulphate was given intramuscularly. The animals were killed one-half hour after

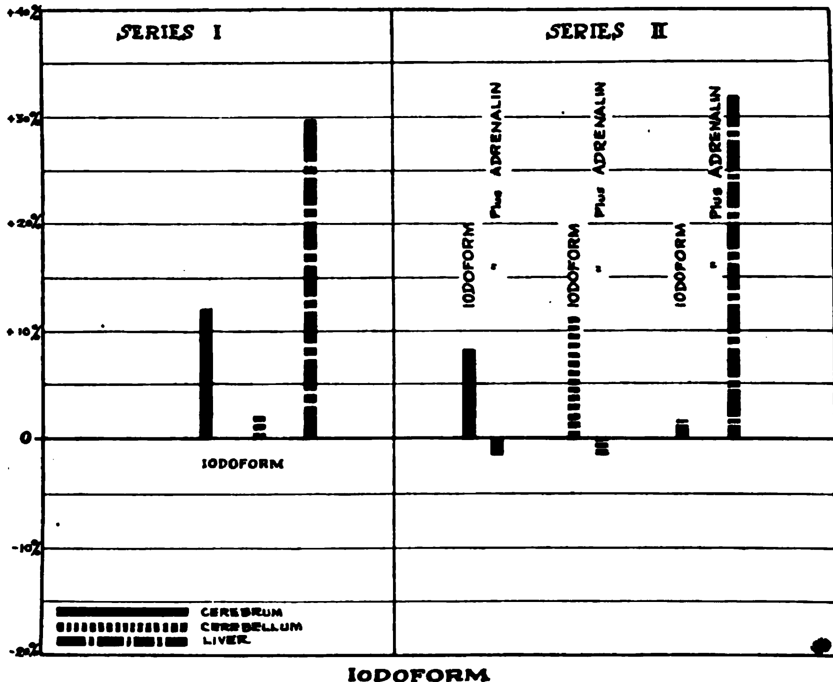


Chart 11. Changes in the electrical conductivity of the brain and of the liver produced by iodoform and by iodoform plus injection of adrenalin. (Percentile variations from the normal.)

the anesthesia was complete and sections taken for conductivity measurements.

To each of another group of four rabbits a like dose of magnesium sulphate was given, followed, one-half hour after anesthesia was complete, by the intravenous injection of 8 cc. (per kgm.) of a 3 per cent

[1] Sollman's *Laboratory Guide in Pharmacology* was used as a guide in determining the dosage in each group of experiments included in this section.

Digitized by Google

TABLE 18

Comparison of the effect of an acid with the effects of an alkali upon the electrical conductivity of the brain and the liver

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL
					per cent				*per cent*				*per cent*
6/11–6/18/19	*Normal VI*	8	*00172*	*1.7*		8	*00131*	*1.0*		6	*00101*	*1.7*	
6/19–6/20/19	Sodium bicarbonate	6	00178	2.2	+ 3.4	6	00139	3.1	+ 6.1	6	00087	6.6	−13.8
1/ 3–2/19/19	*Normal III*	5	*00192*	*1.6*		5	*00162*	*5.3*		5	*00074*	*2.2*	
1/31–2/ 8/19	Hydrochloric acid	9	00168	2.7	−12.5	9	00144	2.7	−11.1	9	00093	4.0	+ 8.1

TABLE 19

Comparison of the effects of magnesium sulphate and of magnesium sulphate plus calcium chloride

DATE OF EXPERIMENT, GROUPS	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL	NUMBER OF ANIMALS	LIVER	AVERAGE DEVIATION PER CENT	PERCENTILE DEVIATION FROM NORMAL
					per cent				*per cent*				*per cent*
2/ 3–2/28/20	*Normal XIII*	*8*	*00179*	*1.9*		*8*	*00126*	*2.3*		*7*	*00063*	*6.3*	
2/19–2/27/20	Mg SO_4	4	00164	1.2	−8.3	3	00130	2.2	+3.1	4	00064	1.7	+ 1.5
	Mg SO_4 + $CaCl_2$	4	00171		−4.4	4	00116	2.1	−7.9	3	00054	5.8	−14.2

Digitized by Google

solution of calcium chloride. In each case the animal became completely conscious almost immediately after the calcium chloride injection. It was killed at once and conductivity measurements made.

The contrasting results in the two series are shown in table 19.

II. Calomel. To each of three rabbits 2 cc. (per kgm.) of a 0.5 per cent solution of calomel in sodium thiosulphate was given hypodermically. The animals were killed 24 to 30 hours later. Each showed marked diuresis and diarrhea, with some salivation (table 20 a).

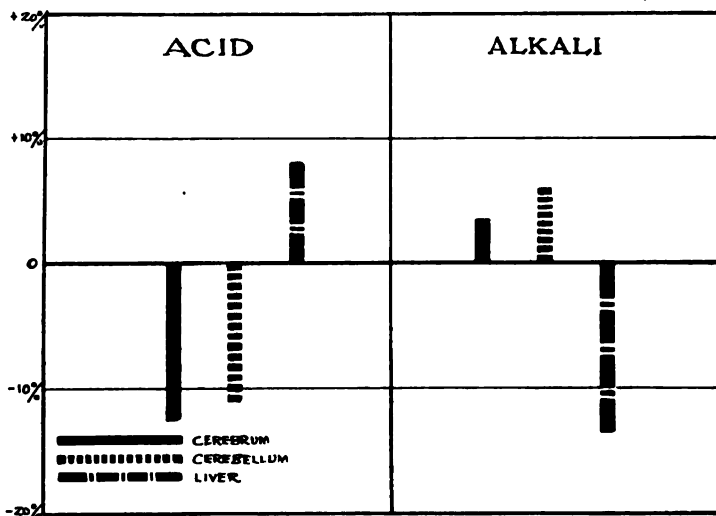


Chart 12. Opposite effects of the injection of an acid and of an alkali on the electrical conductivity of the brain and of the liver. (Percentile variations from the normal.)

III. Caffein. (a) To each of 5 rabbits 4 cc. (per kgm.) of a 1 per cent solution of caffein was given intravenously. Each showed extreme toxic effects and died within 10 to 15 minutes.

(b) To each of four rabbits 1 to 2 cc. (per kgm.) of the 1 per cent caffein solution was given hypodermically in two successive doses about 10 minutes apart, the interval determined by the clinical effects. These showed less marked but positive clinical effects terminating in milder convulsions. The animals were killed when the convulsions appeared.

(c) To each of five rabbits two or three doses as in the last preceding series were given and the animals kept until the following day, when a

Digitized by Google

TABLE 20

Effects of various agents upon the electrical conductivity of the brain

DATE OF EXPERIMENT, GROUP	STIMULUS	NUMBER OF ANIMALS	CEREBRUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL	NUMBER OF ANIMALS	CEREBELLUM	AVERAGE DEVIATION PER CENT	PERCENTILE VARIATION FROM NORMAL
					per cent				per cent
11/ 1-11/15/19	*Normal IX*	*9*	*00181*	*1.3*		*9*	*00135*	*2.2*	
10/28-10/29/19	(a) *Calomel*	3	00166	1.0	− 8.2	3	00136	0.3	+ 0.7
10/29-11/21/19	(b) *Caffein*								
	Single massive dose	4	00163	1.5	− 9.9	4	00.42	1.6	+ 5.1
	Repeated doses								
	1 hour	4	00177	2.2	− 2.2	4	00141	1.5	+ 4.4
	Repeated 2 days	5	00158	1.6	−12.7	5	00.42	3.2	+ 5.1
11/22-12/ 2/19	*Normal X*	4	00179	1.4		5	00128	2.4	
11/19-12/ 6/19	(c) *Sodium bromid*								
	1 hour	3	00184	1.8	+ 2.7	3	00128	4.1	0
	2 hours	3	00180	0.9	+ 0.5	2	00126	0.5	− 1.5
	15-16 hours	2	00180	0	+ 0.5	2	00145	1.1	+13.2
	24 hours	2	00174	4	− 2.7	2	00127	4.6	− 0.7
	48 hours	2	00170	1.4	− 5.02	2	00119	0.6	− 7.03
1/ 6- 1/28/20	*Normal XII*	*5*	*00161*	*4.5*		*5*	*00129*	*3.2*	
1/15- 1/17/20	(d) *Cocaine*								
	Intravenous	4	00167	0.7	+ 3.7	4	00133	1.4	+ 3.1
	Intramuscular	2	00155	2.8	− 3.7	2	00115	1.5	−10.8

Digitized by Google

final dose was given and the animal killed during the resultant convulsion.

The average effects of the caffein upon the electrical conductivity of the animals in each of these groups is shown in table 20 b.

IV. Sodium bromid. Twelve rabbits were divided into 5 groups and given sodium bromid (3 gm. per kgm.) through a stomach tube in single or repeated doses, as follows:

Group 1, 3 rabbits, 1 dose—killed after 1 hour.
Group 2, 3 rabbits 2 doses—1 hour apart—killed 1 hour after last dose.
Group 3, 2 rabbits, 1 dose—killed 15–16 hours later.
Group 4, 2 rabbits, 1 dose on each of two successive days—killed 1 hour after second dose.
Group 5, 2 rabbits, 1 dose on each of 4 successive days—killed 1 hour after last dose.

The average effects upon the electric conductivity of the brains of the animals in each group are shown in table 20 c.

V. Cocaine. (a) To each of 4 rabbits 1.2 cc. (per kgm.) of a 5 per cent solution of cocaine was injected intravenously. The injection was followed by a mild convulsion followed by complete relaxation. The animals were killed in from one-half to three-quarters of an hour after the dose was received.

(b) To each of two rabbits 2 cc. (per kgm.) of a 5 per cent solution of cocaine was injected intramuscularly, and the animals killed one hour later (table 20 d).

Measurements of the electric conductivity of pathological human tissues. In view of the finding of Loeb, Lillie, McClendon and other physical chemists that the permeability of the ovum is increased by fertilization, and the indication of their researches and our own that any alteration in function of the cells is attended by an alteration in their electric conductivity, one would infer that the electric conductivity of tissues in which the cells are in as active a state as in cancerous tissue would be higher than the electric conductivity of normal tissue. We therefore included in our research measurements of the electric conductivity of malignant and benign tumors and of various precancerous conditions.

In this study we have measured 219 sections from 159 clinical cases. These have included malignant and benign tumors of the breast and of the uterus, ulcer and carcinoma of the stomach, carcinoma of the rectum, malignant and benign tumors of the mouth, jaws, and neck, x-ray burns and various types of goiters—hyperplasia, fetal adenoma,

Digitized by Google

multiple adenoma, toxic adenoma, exophthalmic goiter, simple colloid goiter, thyroiditis. Whenever possible adjacent normal tissue has been measured for comparison. The pathological diagnosis and differentiation of different types of tissue in single specimens were made by the pathologist of the surgical section at Lakeside Hospital.

Among the goiters the highest conductivities were found among the adenomata. Variations in conductivity are well illustrated by the following measurements of a goiter, one portion of which was malignant. Three specimens from this gland gave the following measurements:

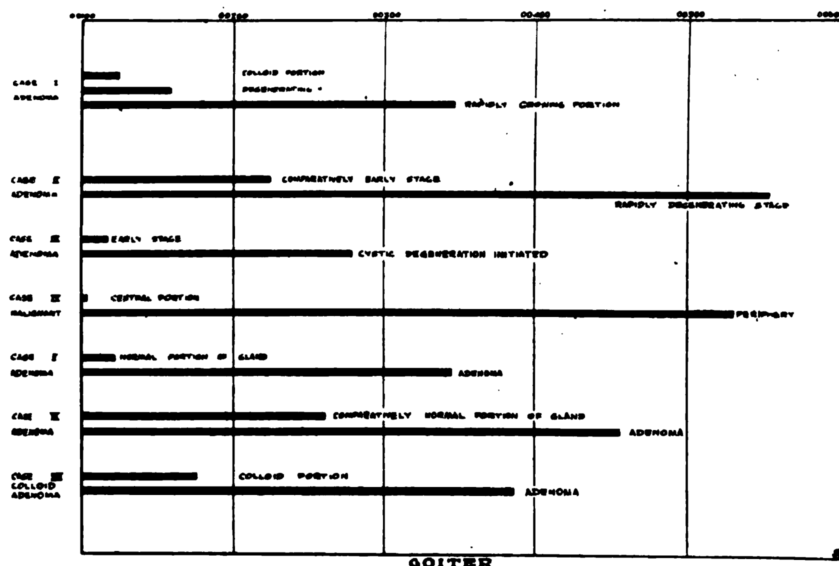


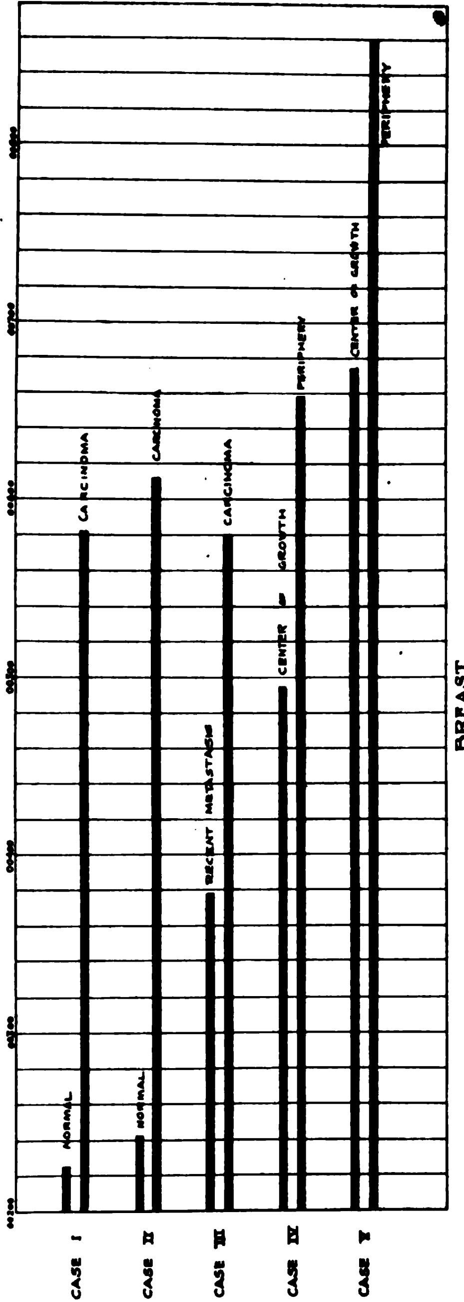
Chart 13. Comparison in seven cases of the electrical conductivity of the comparatively normal or inactive portions of a thyroid gland with the conductivity of the rapidly growing or degenerating portion of the same gland. (Actual measurements.)

colloid portion, 0.00124; early degeneration, 0.00159; rapidily growing portion, 0.00346.

The highest conductivities were found in the degenerating adenomata and the malignant thyroids; the conductivities of the hyperplastic thyroids were lower; and the conductivities of the colloid goiters were the lowest of any of the pathological tissues studied.

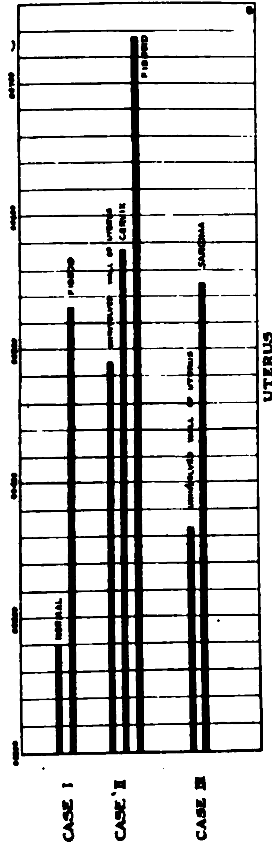
In all instances in which comparative measurements were made the conductivity of the malignant growth was higher than that of a normal portion of the same organ, as is illustrated by the following examples (charts 13 to 15):

Digitized by Google



BREAST

Chart 14. Comparison of the electrical conductivity of normal and of carcinomatous breast tissues and of different stages in the development of carcinoma of the breast. (Actual measurements.)



UTERUS

Chart 15. Comparison in three cases of the electrical conductivity of the comparatively normal portion of the uterus with a tumorous portion of the same uterus. (Actual measurements.)

Digitized by Google

Sarcoma of the uterus:

Comparatively normal portion	0.00367
Sarcomatous portion	0.00550

Mixed tumor of parotid:

Comparatively normal gland	0.00153
Malignant portion (2 sections)	0.00922 0.01058

Carcinoma of the breast:

Comparatively normal portion	0.00225
Carcinomatous portion	0.00581

Carcinoma of the pylorus:

Normal mucosa	0.00126
Base of growth	0.00469

In the light of these comparisons the following measurements of the conductivities of x-ray burns and of the adjacent normal tissues are significant (chart 16).

Case I. Normal tissue, 0.00103; x-ray burn, 0.00717.
Case II. Normal tissue, 0.00151; x-ray burn, 0.00366.

The outer growing parts of cancers showed a high conductivity in contrast with the conductivity of the central non-growing parts.

Carcinoma of the thyroid:

Center of growth	0.00101
Periphery	0.00528

Carcinoma of the uterus:

Center of growth	0.00493
Periphery	0.00658

SUMMARY AND CONCLUSIONS

1. In an attempt to determine the specific electric conductivity of various normal animal tissues and whether or not variations in function are accompanied by measurable changes in their electric conductivity, 4764 sections from 455 rabbits and 219 sections of pathological human tissues have been measured.

2. The specific normal conductivity of the cerebrum, cerebellum and liver can be estimated within a narrow range; while the normal conductivity of other tissues can be estimated within a sufficiently narrow range to determine the order of their relative conductivities.

3. The spinal fluid has the highest conductivity of any of the tissues studied, the lung and the liver the lowest.

Digitized by Google

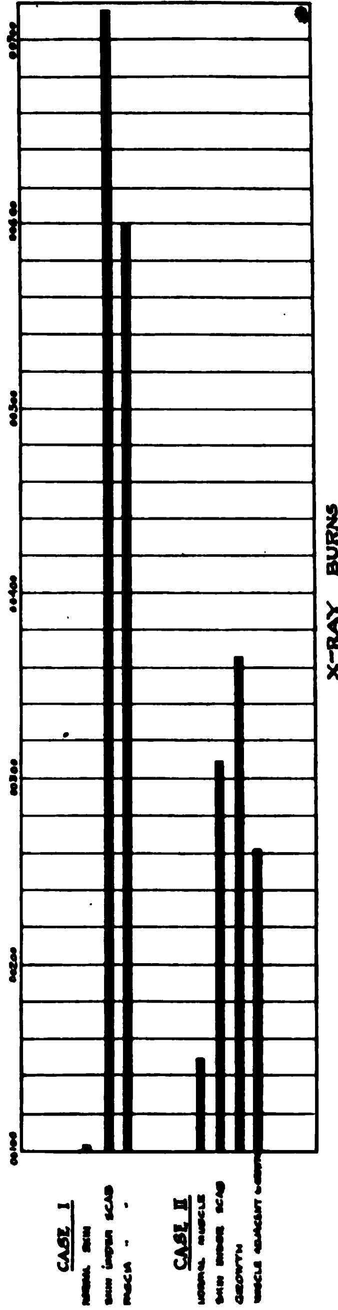


Chart 16. Comparison of the electrical conductivity of normal tissues with tissues involved in x-ray burns. (Actual measurements.)

4. The order of the conductivities of the following tissues was unchanged in all the animals studied with the exception noted in (6); viz., spinal fluid, bile, blood, voluntary muscle, cerebrum, cerebellum, liver, lung. In a limited number of observations the conductivity of the heart fell between that of the cerebellum and the liver, but on account of the wide range of the individual measurements, this cannot be considered as established.

5. The conductivity of normal tissues appears to vary according to the season and the general environment.

6. In every normal adult animal studied the conductivity of the cerebrum was higher than the conductivity of the cerebellum. In fetuses and in very young rabbits this relation was reversed—the conductivity of the cerebellum being higher than the conductivity of the cerebrum until about the time when the young rabbit left the nest and began voluntary activities, when the normal adult conductivity relation of the cerebrum and the cerebellum appeared to be established. A most significant corollary to this observation was found in the post-mortem examination of the brains of two patients, one of whom died after days of unconsciousness resulting from a brain tumor, while the other who died from carcinoma of the stomach, was conscious to the end. In the patient who had been unconscious, *the conductivity of the cerebellum was higher than that of the cerebrum.* In the other patient, as in all our normal animals, the conductivity of the cerebrum was higher than that of the cerebellum.

7. The conductivity of the gray matter of the brain is higher than that of the white matter.

8. Exhaustion from any cause—surgical shock, insomnia, emotion (fright), infection, etc.—is marked by a *diminished conductivity of the brain* and an increased conductivity of the liver.

9. The immediate effect of activation appears to be an increased conductivity of the brain, tending later to decrease as the stage of exhaustion approaches. As the charts indicate, this has been shown to be an immediate effect of physical injury; an early effect of the injection of diphtheria toxin; an immediate effect of the injection of adrenalin.

10. Thyroid feeding in large doses over a prolonged period produces the typical symptoms of hyperthyroidism with ultimate exhaustion accompanied by the changes in the conductivity of the brain typical of exhaustion from any other cause; i.e., the conductivity of the cerebrum and cerebellum is decreased.

Digitized by Google

11. Thyroid feeding in moderate doses until the symptoms of hyperthyroidism appear but not to the stage of exhaustion produces conductivity changes in the brain typical of the stage of stimulation produced by other agents; i.e., increased conductivity of the brain and decreased conductivity of the liver. These changes were diminished or reversed by the administration of adrenalin.

12. Iodoform increases the conductivity of the brain and the liver. These changes are reversed by adrenalin.

13. The injection of hydrochloric acid produced *diminished conductivity of the cerebellum and cerebrum and increased conductivity of the liver*. The injection of sodium bicarbonate produced *increased conductivity of the cerebellum and cerebrum and decreased conductivity of the liver*.

14. Rabbits were kept awake continuously for 96 hours. At the end of this period a number were killed and conductivity measurements made; others were allowed a brief period of rest of from four days to a week. At the end of the insomnia period the *conductivity of the brain was decreased* and the conductivity of the liver was increased; at the end of the short period of rest, the conductivity of the brain and of the liver was but little changed, if at all; at the end of the longer periods of rest, the brain was again approaching its normal conductivity.

15. A limited number of observations indicate that the changes produced by infection are minimised provided the infection is applied in the presence of morphin: that is, excessive infection alone decreases the conductivity of the brain and increases the conductivity of the liver; in this limited series of observations the conductivity of the brain remained practically unchanged when diphtheria toxin was administered in a morphinised animal, and the conductivity of the liver was but slightly altered.

16. A limited series of observations of the influence upon the electric conductivity of the brains of animals of various agents, which produce marked clinical effects, indicate that the progress of alteration in function produced by any agent is coincident with changes in electric conductivity.

17. In the pathological specimens studied, active malignant growths have a high conductivity in comparison with adjacent normal tissue and the inactive portions of the same growth, and with growths of a non-malignant type.

Digitized by Google

GENERAL CONCLUSIONS

1. Influences which affect the general physical condition of the organism produce changes in electric conductivity in the dominating reactive tissues, these changes being uniformly and measurably manifested in the brain and the liver. Apparently these changes in conductivity appear more promptly than any gross clinical alteration.

2. Apparently the liver is more promptly and more markedly affected than any other tissue, as animals showing either no or very slight changes in the cerebrum and cerebellum will often show a marked alteration in the conductivity of the liver. On account of the wide variation in liver measurements and the apparent susceptibility of this organ to seasonal and environmental changes, the effects of applied agents are best determined by measurements of the cerebrum and cerebellum.

3. A study of the individual measurements from which the averages have been computed seems to indicate that the variations represent slightly different stages in a process that varies in rate in different animals and in the different organs of the same animal.

4. In view of the above indication and the direct evidence of the measurements we feel justified in the assumption that the first effect of stimulation within the organism is a slight decrease of the conductivity of the liver followed by a rapid continuous rise to above the normal as the state of exhaustion approaches; a slight and prompt increase in the conductivity of the cerebellum followed by a gradual continuous fall; a relatively slower increase in the conductivity of the cerebrum followed by a gradual continuous decrease.

5. These studies indicate that electric conductivity measurements provide a means whereby to further the interpretation of the normal operation of the organism, and whereby to measure the progress of pathological processes within the various organs and tissues.

6. From our findings to date, it would appear that the *intracellular changes in exhaustion and shock which are revealed by the microscope are paralleled by alterations in electric conductivity, and that both the histologic and the electric changes bear a direct relation to the vitality of the organ.*

BIBLIOGRAPHY

(1) Lillie: Biol. Bull., 1909, xvii, 188; Science, N. S., 1909, xxx, 245; This Journal, 1916, xli, 126; Journ. Gen. Physiol., 1920, iii, 107.

(2) Loeb: Journ. Biol. Chem., 1916, xxviii, 175; The organism as a whole from a physico-chemical viewpoint, 1917.

Digitized by Google

(3) Osterhout: Science, 1911, xxxiv, 187; Science, 1912, xxxv, 112; Science, 1912, xxxvi, 350; Bot. Gaz., 1913, lv, 446; Science, 1913, xxxvii, 111; Journ. Biol. Chem., 1917, xxxi, 585; Journ. Gen. Physiol., 1919, i, 515; Journ. Gen. Physiol., 1919, 405.
(4) Mathews: Physiological chemistry, 1916.
(5) McClendon: Science, 1914, xl, 70, 214; This Journal, 1915, xxxviii, 173; Physical chemistry of vital phenomena, 1917.
(6) Hill: Journ. Physiol., 1910, xl, 190.
(7) Lucas: Journ. Physiol., 1906, xxxiv, 372; Journ. Physiol., 1907, xxxvi, 253; Journ. Physiol., 1908, xxvii, 112; Journ. Physiol., 1910, xl, 225; Proc. Roy. Soc., 1912, lxxxv B, 495.
(8) Carlson: This Journal, 1911, xxvii, 323.
Crehore, and Williams: Proc. Soc. Exper. Biol. and Med., 1913, xi, 58.
Gotch: Journ. Physiol., 1902, xxviii, 275.
Gotch and Burch: Proc. Physiol. Soc. London, 1898, 22.
Gotch and Horsley: Phil. Trans., 1891, clxxxii, 267.
Harvey: Journ. Exper. Zoöl., 1911, x, 507.
Kite: Biol. Bull., 1913, xxv, 1; This Journal, 1913, xxxii, 146.
Waller: Journ. Linnaean Soc., 1904, xxxvii, 32; Rep. Brit. Ass. Adv. Sci., 1913, 24.
(9) Galeotti: Zeitschr. Biol., 1902, xliii, 289; Zeitschr. Biol., 1904, xlv, 65.

Digitized by Google

STUDIES ON THE EFFECT OF DIET ON THE WEIGHT OF THE HYPOPHYSIS AND THYROID GLAND OF THE ALBINO RAT, AND ON THE ACTION OF THEIR EXTRACTS ON THE ISOLATED SMALL INTESTINE

LYDA MAY DEGENER

From The Wistar Institute of Anatomy and Biology

Received for publication November 8, 1921

This research was undertaken to determine whether alterations in weight of the thyroid and hypophysis and in the effects of their extracts on the isolated intestine could be brought about by different diets. In the previous studies made by Watson (13) and others, the observers were mainly concerned with the histological changes, and no study was made of the extracts from the glands. My conclusions are based on several series of observations. In the first series it was planned to test three diets characterized by oatmeal, vegetables and meat respectively. Later, in a second series, diets were included in which potassium iodide and "thyroxin" were administered to the test animals.

Material and methods. The rats were secured from the colony of The Wistar Institute. Two carefully selected litters of the same age were taken for each experiment. An equal number of rats from each litter was placed in the control and test cages, the sexes being kept separate. The ages and weights at the first feeding of the special diet were recorded. Weekly weighings were then made until the last animal had been killed. Tables 1 to 5 give in a condensed form the weights and other data.

At varying intervals during each experiment a rat from the test lot, with its accompanying control, was weighed, then killed by ether, measured, and the viscera removed. The weights of the thyroid, brain and hypophysis were taken separately and the percentage of water in the brain was determined. The thyroid and hypophysis were ground separately and extracted. The details of this last procedure will be given later.

The diets used: Oatmeal diet. The test diet was begun on September 30, 1919, in the form of steamed oatmeal and milk. The group com-

Digitized by Google

prised four females and six males, 27 days old. The animals throve and grew on this diet, although some cases of pneumonia appeared among both tests and controls. The experiment was continued from 105 to 117 days. Table 1 gives the data for this group.

Vegetable diet. This diet was begun on September 30, 1919. The rats seemed to be too young (27 days) for the test, as they showed symptoms of ill health. They were returned to the control diet on October 4 but on November 4 the test diet was resumed. This consisted of

TABLE 1

Oatmeal diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	3 T.	36	158	20.6	4.8	1.516	78.5	105	131
♂	3 C.	36	177	17.7	5.4	1.633	78.3	105	133
♀	2 T.	32	150	15.2	8.6	1.617	77.9	117	145
♀	2 C.	39	133	17.2	7.0	1.575	78.5	117	143

T = tests; C = controls.

TABLE 2

Vegetable diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	1 T.	113	228	22.6	6.5	1.734	*75.4	108	133
♂	2 C.	93	199	16.9	5.9	1.657	78.2	108	134
♀	4 T.	73	136	17.0	6.1	1.540	78.3	113	139
♀	4 C.	76	141	15.8	7.2	1.555	78.4	113	139

* This value is abnormal.

all green vegetables with occasional carrots, turnips, potatoes (steamed and raw), wheat, grape-fruit skins and beans. With the exception of a single male, all of the test rats were below the standard weight. Not only were they smaller in size, but were also obviously frail. They were less tame than the rats in other groups and toward the end of the experiment this reaction became even more noticeable (table 2).

Meat diet (a). This experiment was begun September 30, 1919, when the rats were 28 days old and had just been taken from the mother.

Digitized by Google

Steamed beef was fed twice a day. The rats became peeked, listless and inactive. They were cyanosed and cold to the touch. One rat was eaten by its brothers, despite the meat diet. On October 3 they were put on diet consisting of warm milk and condensed milk and wheat. On this they recovered, and were returned to the test diet on November 1. Raw beef was given from this date on, in preference to the steamed beef. Throughout the remaining period of the experiment, i.e., up to March 26, 1920, all were in splendid condition, always hungry, tame and

TABLE 3a

Series I. Meat

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	5 T.	24	226	19.4	7.7	1.836	78.2	125	185
	5 C.	23	232	17.0	7.0	1.837	78.0	125	186
♀	3 T.	24	169	18.2	9.2	1.771	78.2	109	168
	3 C.	24	165	17.6	9.6	1.737	78.3	109	169

TABLE 3b

Series II. Meat

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	3 T.	143	183	15.4	5.9	1.789	78.4	27	104
	3 C.	135	193	15.9	5.8	1.799	78.7	27	104
♀	3 T.	119	148	15.0	8.5	1.651	78.1	29	119
	3 C.	109	144	13.5	9.1	1.629	78.3	29	119

playful. The fur on the test animals was especially silky, soft and white, and the good condition of the group was striking. In three cases the lungs were found to be slightly infected (table 3 a).

A second series of observations was made to control this experiment with meat, and also to replace the first experiments made with potassium iodide and with "thyroxin," both of which had failed through accidents. The rats in these groups ranged from 76 to 98 days of age at the commencement of the test, and the experiments lasted from 14 to 37 days. Tables 3b, 4 and 5 give the data.

Digitized by Google

Meat diet (*b*). These rats were in excellent health; in two cases however the lungs were slightly infected. At each feeding they were given lean beef until entirely satisfied. Half of these rats were up to or above the standard weight (table 3 b).

Potassium iodide with normal diet. The first series tested has been omitted from the records because of serious lung infections and we have merely noted the effect of the lung infection on the water content of the brain.

In the second series a group was run for 26 to 32 days; the rats ranging in age from 83 to 120 days (table 4). The amount of KI given was 7 mgm. to 100 grams of body weight. In this group five of the twelve animals showed a light lung infection, but this was not severe enough to influence the percentage of water in the brain. The body

TABLE 4

Series II. Potassium iodide with normal diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	2 T.	144	174	15.0	6.0	1.781	78.4	26	120
	2 C.	137	202	19.4	6.7	1.833	78.7	26	120
♀	4 T.	119	137	15.0	7.8	1.772	78.1	32	115
	4 C.	115	147	13.9	8.2	1.793	78.3	32	115

weights were low. The health of the rats was good and no general effect of the KI was evident.

Thyroxin with normal diet. This material, first prepared by Doctor Kendall (5) and Kendall and Osterberg (6) was obtained from E. R. Squibb & Sons in the form of 0.2 mgm. tablets. One tablet was ground into fine powder and given each test rat at each feeding.

Each animal was kept in a separate cage in order to check the amount of food eaten. In the first group thus treated the controls failed to grow properly and the data have been discarded. A second group was therefore treated in the same manner. Table 5 gives the data for the second group. These rats remained in excellent health and more than half of the test rats were up to or above the standard weight. Two cases of slight lung infection were noted at autopsy (table 5).

Comments on weights. In all these groups (tables 1 to 5) the average body weights are low for the age in both the control and test animals,

Digitized by Google

and the weights of the organs are low for the body weights when compared with the standard values.

The discussion on the weights will be confined to a comparison of the tests with the controls for each sex, the age being the same and the animals compared being always members of the same litter. The data in the tables make twelve such comparisons possible.

As the weights of the thyroid and hypophysis are correlated with the body weight, it is necessary in comparing the weights for the test group with those for the controls to take the body weights into consideration. It is also necessary to keep in mind that the variability in the weights of these glands is high.

The procedure followed was to find from the appropriate table in *The Rat* (1) the percentage difference in the weights of the glands (thyroid and hypophysis) as there given according to the observed

TABLE 5

Series II. Thyroxin with normal diet

SEX	NUMBER	INITIAL WEIGHT	FINAL WEIGHT	THYROID WEIGHT	HYPOPHYSIS WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	DAYS OF EXPERIMENT	AGE AT KILLING
		grams	*grams*	*mgm.*	*mgm.*	*grams*	*per cent*		
♂	3 T.	133	187	17.4	6.0	1 863	78.1	27	111
	3 C.	129	224	16.1	6.2	1.877	78.3	27	111
♀	3 T.	138	169	13.8	8.5	1.793	78.6	28	118
	3 C.	128	169	15.5	9.0	1.727	78.5	28	118

body weights for each sex, and then to compare this with the corresponding percentage difference between the values for tests and controls in our records. If these two determinations agreed within 5 per cent, plus or minus, we concluded that the experimental conditions had not modified the weight of the organ.

When the data in tables 1 to 5 are treated in this manner there is some indication that in the "vegetable" group, table 2, the weight of the thyroid is increased and the weight of the hypophysis diminished, and in the "meat" group (tables 3a and 3b) that the weight of the thyroid is somewhat increased. In the other six pairs there is no difference shown by this method which can be attributed to diet. As the results are mainly negative, it is not deemed necessary to present the figures giving these relations.

For the body weights the brain weights are low. On the average the tests and the controls show nearly the same relative brain weight.

Digitized by Google

The dietetic treatment given these rats has not therefore modified the weight of the brain. When the percentage of water in the brain—on age—is compared in the tests with that in the controls, it is found to agree perfectly. Consequently the myelination process also has not been modified (2).

As previously noted, in a group of ten rats given potassium iodide for 60 days, all the animals were found to have severe lung infection and the data on weight were therefore excluded from the tables.

It was of interest to note however that in this group the percentage of water in the brain was 77.5 for the control rats and 77.6 for the tests. The percentage of water to be expected on age was 78.14 (1, table 74). Similarly, in the discarded group treated with thyroxin, but also suffering from severe lung infection, the percentage of water in the controls was 77.7 and in the tests 77.6. According to age 78.15 per cent was to be expected. The differences therefore are 0.6 and 0.5 per cent respectively.

These results agree with the observations of King (7) which show that severe lung infection in the mature rat reduces the percentage of water in the brain about 0.5 per cent.

Preparation of material for the study of the extracts. A test and control rat of the same sex were taken from a group and each weighed. The several organs were then removed and weighed. The thyroid, from which the parathyroids had not been removed, and the hypophysis were then ground separately with a small quantity of white Berkshire sand and mixed with Tyrode's solution. The extract thus obtained had a concentration of 0.25 per cent. The four test tubes containing the extracts from the thyroid and the hypophysis, test and control respectively, were put in the water bath (39°C.) for 2 hours.

An extra male rat was killed by crushing the cervical cord under ether, and a piece of the duodenal intestine was removed. This was placed immediately in a dish containing oxygenated Tyrode's solution and kept at 39°C. The intestinal strip was then cut lengthwise, freed from mesenteric tissue and a piece approximately 1.5 cm. prepared. This was attached at each end by passing through it a needle threaded with white silk, and suspended vertically in a glass tube filled with Tyrode's solution. The response of the strip could thus be recorded as described by Hatai and Hammett (4).

Action of extracts. In some cases after the strip had been immersed in Tyrode's solution the pulsations were for a time rapid and the tonus high. Occasionally it remained inactive. Usually therefore the in-

Digitized by Google

testine was left for 10 minutes in order that it might become normal in behavior. Sometimes it was necessary to initiate the pulsations by mechanical stimulation.

The general conditions which modify the response of the strip have been stated by Hatai and Hammett (4). One condition is sex and another is age. In accordance with the previous work, the observations here reported are only those made on the intestines of male rats 150 or more days of age.

The rat from which the intestine was taken had been kept without food for from 12 to 24 hours previous to the experiment. Before applying the extract the intestine was washed two or three times with the Tyrode's solution, and after returning to the normal beat the gland extract was added. Two cubic centimeters of the thyroid extract were used, but the hypophysis extract was added in proportion to the quantity on hand.

As pointed out and emphasized by Hammett and Tokuda (3) it is probably not the characteristic secretion of these glands in the extracts which causes the response of the intestinal strip, but something else.

The results according to diet may be summarized as follows:

Thyroid. For the extracts of the thyroid there was no clearly marked difference in the response to the test extract as compared with that to the control in the case of any of the diets here used.

Hypophysis. In the case of the extract from the hypophysis differences did occur as shown in charts 1 and 2.

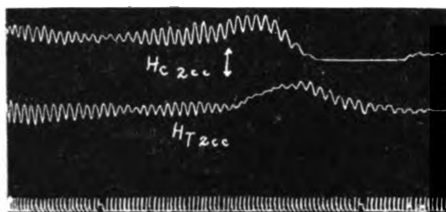
Comments on the action of the extracts: Hypophysis extract. The control extract causes a relaxation of the strip in every case. This is sometimes preceded by a slight contraction (chart 1). The test extract in the oatmeal and vegetable diets causes a marked contraction (charts 1 and 2), but in the meat, the potassium iodide and the thyroxin groups, the reaction for the test extract is similar to that for the control (chart 3 for thyroxin).

It appears possible therefore that the effect of the extract of the hypophysis has been modified by the diet in the oatmeal and vegetable groups.

To obtain an explanation of this difference in behavior, some observations were made on the action of the glandular and nervous portions of the hypophysis taken separately.

In the first instance ten adult male rats were used, and the average body weight calculated. The rats were killed in the usual manner, the hypophysis removed, the ten glandular portions separated from the ten

Digitized by Google



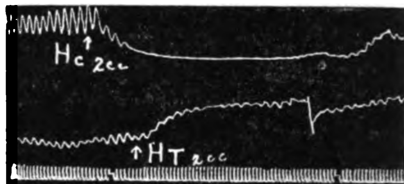


Chart 1 Chart 2

Chart 1. Oatmeal diet. Hypophyseal extract—From two female albino rats 144 days old. *H. C.* Control extract. *H. T.* Test extract.

Chart 2. Vegetable diet. Hypophyseal extract from two male albino rats 133 days old. *H. C.* Control extract. *H. T.* Test extract.

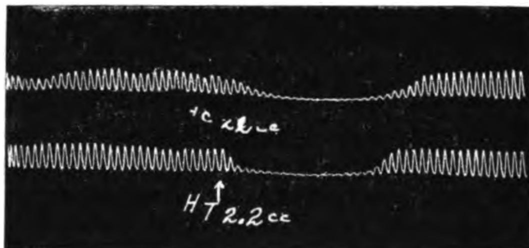


Chart 3. Standard diet with thyroxin (0.2 mgm. thyroxin). Hypophyseal extract from two male albino rats 97 days old. *H. C.* Control extract.. *H. T.* Test extract.

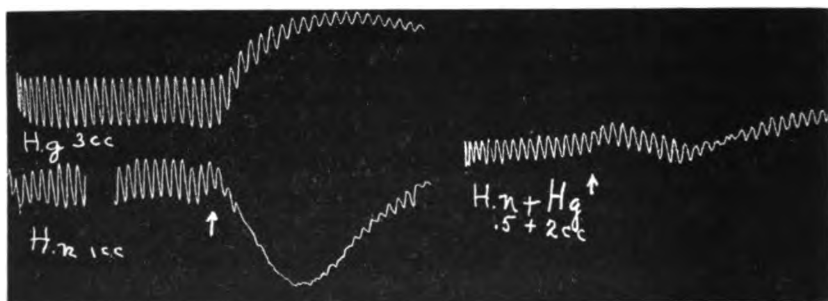


Chart 4 (a and b). Effects of extracts of parts of hypophysis—separately and combined, on the intestinal strip. (a) *H. g.* Extract of glandular portion alone. *H. n.* Extract of nervous portion alone. (b) *H. n.* and *H. g.* Combined in normal proportions.

Digitized by Google

nervous portions, and weighed separately. The values obtained are given in table 6. The nervous and glandular lobes were ground separately and the extracts tested on a piece of intestine from a male rat. The preparations were made in the morning and tested at 2 p.m. The amount of solution from the nervous portion was 5 cc. and that from the glandular 22.4 cc. According then to this ratio the extracts were tested on the intestine, first separately as nervous and glandular extracts, and then combined in the same proportion, as the extract from one rat. Chart 4 illustrates the results obtained. To determine any difference between the glands from males and females, ten females were taken and the glands treated in the same manner. The glandular and nervous portions, after being ground and extracted, were tested on the intestine from a *female* rat. The results were similar to those in chart 4, yet the curve from the nervous portion does not show as great a relaxation.

TABLE 6

Average weights of nervous and glandular lobes of hypophysis from rats used in tests

RATS	BODY WEIGHT	GLANDULAR	NERVOUS	TOTAL WEIGHT OF HYPOPHYSIS
	grams	*mgm.*	*mgm.*	*mgm.*
10♂	229	5.6	1.24	6.8
10♀	167	6.2	1.18	7.4
5♂	193	4.9	1.24	6.0
5♀	125	4.8	1.00	5.8

As a second check, five females and five males were treated as above, and the extracts tested first on a male intestine and then on female intestine to determine the differences in response. For the weights of these lobes see table 6.

The male and female glandular extracts gave similar results when tested on the male intestine, but the female nervous extract caused a greater relaxation than did the corresponding male extract. When the male and female glandular extracts were tested on female intestine, essentially similar results were obtained throughout.

Since the two parts of the hypophysis yield extracts which influence in opposite ways the intestinal strip, it is possible to interpret our results in the light of this relation.

In the normal reaction to the control hypophysis extract, the effect of the nervous portion predominates and the strip relaxes. When, on the other hand, the intestine contracts under the influence of the total

Digitized by Google

hypophysis extract, it means that the reaction characteristic for the glandular portion is predominating. At the moment, however, it is not possible to determine whether this predominance is due to an increase in the active substance characteristic for the glandular portion, or a decrease in that characteristic for the nervous portion of the gland.

DISCUSSION

There are several studies on these glands in rats and the results may be briefly considered according to the diet used.

Oatmeal. Watson (12) took six young rats, 6 weeks old, and fed them for from 4 to 8 weeks on a diet of uncooked oatmeal and water. An equal number of controls was fed bread and milk. He states that the thyroid was greatly enlarged and histologically gave evidence of greatly increased functional activity. A similar result had been obtained on an exclusive porridge diet made with skimmed milk (14). The average weights given by Watson in his first series are: test rats, thyroid 0.078 gram and controls, 0.029 gram.

As indicated in table 1, my test thyroids are on the average only very slightly heavier than the controls, thus showing much less difference than was obtained by Watson.

Vegetable diet. Slonaker (10) in his experiments with a vegetable diet on albino rats found the test rats to age more quickly, to be retarded in their growth, and on the whole frail and weak. The general appearance of my test rats checks with that of his series. Thus, with but one exception, a vigorous test male, all my test animals on this diet were considerably below the standard weight and in a poor physical state. Nevertheless the test thyroids appeared relatively somewhat heavier (table 2).

Meat diet. Watson (11) and Watson and Hunter (14), in experiments with an exclusive diet of ox flesh, found that the young rats did not do well, but with older rats particularly fine animals were obtained. Watson found in young rats on a meat diet distinct histological changes in the thyroid gland with wide variations in the amount of colloid and the secreting cells. These changes were frequent, but not constant, and were most pronounced in animals which did not gain normally in weight. As my tables 3a and 3b show, my general results were similar to those obtained by Watson.

Potassium iodide. Following intravenous injection of potassium iodide, Marine and Rogoff (9) found that iodine was taken up by the thyroids almost at once. The amount of absorption was correlated

Digitized by Google

with the functional state of the gland. So rapid was the absorption that practically none of the iodine was excreted. In my tests KI given with the food does not appear to produce any marked change in the size of the thyroid or the physiological effects of the extract. Kojima (8) notes, however, that administration of potassium iodide causes an accumulation of colloid material in the rat thyroid.

As to the effect of diet on the hypophysis, I do not find any observations which bear on the present problem.

SUMMARY

Feeding experiments with five diets were conducted on 200 albino rats, ranging in age from 27 to 250 days and fed for periods from 10 to 175 days upon *a*, oatmeal and milk; *b*, vegetables; *c*, meat; *d*, standard diet plus potassium iodide; and *e*, standard diet plus "thyroxin."

Observations were made on the effect of each diet on *a*, body weight; *b*, weights of the thyroid and hypophysis; and *c*, the physiological effect of the extracts of these glands on the isolated intestine. In addition, the action of the extracts of the parts of the hypophysis on the isolated intestine was determined and the effect of the diets on the brain weight and water content of the brain noted.

Body weight. The body weights in all the groups were low for the age in both control and test animals. There is but a slight difference in the final weights of the tests and controls. The controls were on the average heavier by 5 grams.

Weights of the glands. The normal variability in the weights of both the thyroid and hypophysis is large, and moreover these weights are correlated with the body weights. When compared with the standard tables, all the glandular weights were low for the body weights. Taking the averages, and recording the difference of the tests from the controls, the oatmeal thyroids were heavier than the controls by 1 mgm., the hypophysis by 2 mgm. In the vegetable group the thyroid was heavier by 4 mgm., a result mainly due to one unusually large male test rat. The hypophysis was lighter by 1 mgm. In the meat group the weight of the thyroid was somewhat greater, but that of the hypophysis not altered. In the potassium iodide and thyroxin groups there is practically no modification in the weights of the test glands.

Activity of the gland extracts: Thyroid. For the thyroid no definite difference in the effects of the control and test extracts could be made out in any of the diet groups.

Digitized by Google

Hypophysis. In the case of the hypophysis the test extract causes a contraction of the intestinal strip in the oatmeal and the vegetable diet groups. The control extract always causes relaxation.

Action of the extracts from parts of the hypophysis. The responses to the extracts from the nervous and glandular lobes of the hypophysis are opposed to each other. The glandular lobe extract causes a contraction and the nervous lobe extract a relaxation. The ratio of the weights of the nervous to the glandular lobes is 1 to 4 and when extracts from both lobes are combined in this proportion, the reaction obtained is intermediate or similar to that of the entire hypophysis. This suggests an explanation for the results found in the oatmeal and vegetable groups, in which for some reason the effect of the glandular portion predominated.

Brain weight and percentage of water. The brain weights are low for the body weights, but the relative weights in the tests and controls are similar. The several diets have not therefore modified the weight of the brain, and the percentage of water in the brains of the test and control series agrees perfectly. However, in two groups with severely infected lungs (groups not otherwise used) the percentage of water on age was low. This effect of lung disease on the percentage of water has been noted already by King (7).

BIBLIOGRAPHY

(1) DONALDSON: 1915 The Rat. Data and reference tables. Memoirs of The Wistar Institute of Anatomy and Biology, no. 6, 1915.
(2) DONALDSON: Journ. Comp. Neurol., 1916, xxvi.
(3) HAMMETT AND TOKUDA: This Journal, 1921, lvi, 380.
(4) HATAI AND HAMMETT: This Journal, 1920, liii.
(5) KENDALL: Journ. Biol. Chem., 1919, xxxix.
(6) KENDALL AND OSTERBERG: Journ. Biol. Chem., 1919, xl.
(7) KING: Journ. Comp. Neurol., 1911, xxi.
(8) KOJIMA: Quart. Journ. Exper. Physiol., 1917, xi.
(9) MARINE AND ROGOFF: Journ. Pharm. Exper. Therap., 1916, viii.
(10) SLONAKER: Leland Stanford Univ., Cal., 1912.
(11) WATSON: Journ. Physiol., 1904, xxxii.
(12) WATSON: British Med. Journ., 1907, 1.
(13) WATSON: The influence of diet on the structure of the tissues—Reprinted from *Food and feeding in health and disease.* Oliver & Boyd, Edinburgh, London, 1910.
(14) WATSON AND HUNTER: Journ. Physiol., 1906, xxxiv.

Digitized by Google

VARIATIONS IN OUTPUT OF BILE SALTS AND PIGMENTS DURING 24-HOUR PERIODS

OBSERVATIONS ON STANDARD BILE FISTULA DOGS

F. P. WISNER AND G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication November 19, 1921

In earlier communications from this laboratory (1), (2) have been reported many series of observations dealing with the physiology of the bile pigments and bile salts in healthy bile fistula dogs. It has been shown for example that the bile pigments can be modified at will by diet factors and many agencies which modify liver function (1). The bile pigment output therefore is varied by the constructive activity of the liver as well as by the commonly accepted disintegration of hemoglobin in the body. So too the bile acids or salts in bile fistula bile may be modified readily and promptly by diet factors (3). It is easy to demonstrate complete dissociation of these two activities of the liver showing that bile pigment production is a totally different function of the liver and separated completely from the bile salt production.

In all of our earlier experiments and as a routine we used unit daily collections of 6 hours. We felt that this period of 6 hours could be tolerated easily by a healthy dog and that the general condition of the dog was a very important element in the experiment. We have been able to maintain in perfect health many dogs on this daily routine. Some of our bile fistula animals have lived in perfect health for 2 to 4 years. We felt that the 6-hour unit collection made at the same time each day, preceded and followed by exercise, together with regular feeding hours, would be truly representative of the whole day's output. Many other workers have used 24-hour periods and we have reviewed this work elsewhere (4). In general these long collection periods tend to produce abnormalities in the dogs and the results may therefore be open to just criticism.

For our own information, we felt that it was necessary to make several 24-hour collection periods on our standard dogs. We believe these data

Digitized by Google

for 24-hour experiments are more satisfactory than any available in the literature. Moreover, it enables us to correlate our published work with some of the experiments of the older investigators. The routine given below does not cause impairment of health and was carried out with much care as to detail. The dogs were not upset by the procedure and were comfortable during the entire 24-hour period. Many other experiments of similar type were carried out but are not included here as the tabulated experiments are truly representative.

Methods. The method of bile fistula collection has been described elsewhere (4). So too the operative procedure and general hygienic routine have been reviewed (4). The *daily routine* for these experiments was as follows: The regular 6-hour collection was made for the first 4 days of the week. This daily routine consisted of yard exercise followed by a preliminary drainage period of 30 minutes which allowed the escape of any excess of night bile. In some dogs there is an accumulation of bile during the night owing to slight obstruction in the fistula when the collection tube is not in place. This causes more or less dilatation of the bile passages and is not a desirable factor. The regular 6-hour collection follows this preliminary drainage. Dogs are fed 2 hours after this collection is started unless note is made to the contrary. At the end of the collection, after the collection binders have been removed, the dogs are exercised, fed and locked in their cages.

On the fifth day of the week, the dogs were put through the regular routine, except that at 4 p.m., instead of taking off the binders, removing the drainage tubes and putting the dogs away in their cages for the night, a second collection period was started. This second period lasted from 4:00 to 10:00 p.m. At about 4:00 p.m., the dogs were transferred to their cages and were kept in an upright position by specially prepared night binders. These consisted of canvas slings suspended from the tops of the cages and provided with four holes for the dog's legs and an additional hole for the drainage tube. These binders were suspended at such a height that the dogs could either stand up, or rest the weight of their bodies on the binders by a slight flexion of their legs. Rests were provided for the dogs' heads, so that they could sleep comfortably in a semi-standing position. A third collection period extended from 10 p.m. to 4 a.m. on the following day and a fourth period lasted from 4 a.m. to 10 a.m., thus completing the 24-hour drainage and securing the day's output of bile in four 6-hour samples. The weekly routine thus consisted of a single 6-hour collection on the first four days of the week, followed by four consecutive 6-hour collections on the fifth day. The dogs were then rested till the following Monday.

Digitized by Google

Analysis of bile. The specimens of bile secured were analyzed for their content of bile acids and bile pigments. These methods have been fully described elsewhere in the publications of this laboratory. In brief, the bile acid method (5) consists in precipitation of proteins by alcohol, followed by an estimation of amino nitrogen present in the unhydrolyzed sample. A sample of the alcoholic filtrate is then hydrolyzed by means of sodium hydrate and its content of amino nitrogen estimated. This, minus the amount of amino nitrogen present in the unhydrolyzed specimen represents the amino nitrogen held in combination as taurocholic acid. The amount of taurocholic acid is estimated from the amino nitrogen by multiplying by the factor 36.72. Taurocholic acid is the only bile acid found in dog's bile.

The bile pigment method consists, in brief, of the following procedure. A 1 to 50 or weaker dilution of the bile in acid alcohol is filtered and allowed to stand in a flask for 24 hours, or until the pigment is converted to a blue green color. The solution is then read against a standard color wedge (4). The pigment readings are all expressed in terms of milligrams of bilirubin.

Feeding of dogs. The diet of the dogs, on the day of the experiments, was carefully controlled. At night the animals were always kept in metabolism cages, with only water before them. In the day time all food was excluded except the particular diet upon which the dogs were being kept, and they were fed at stated times twice a day. The amounts of food ingested were regularly recorded. Over the week-ends the dogs were rested on a mixed diet.

The carbohydrate diet referred to in the tables consisted of a mixture of potatoes, rice and milk, in the following proportions: potatoes, 40 per cent; rice, 28 per cent; milk, 32 per cent.

The meat diet consisted of meat alone. On most occasions extracted veal, obtained from the media kitchen, was used; but occasionally scraps of roast beef from the hospital kitchen were fed. On the days when mixed diet was used, the amounts ingested were not recorded. The mixed diet of course varied in content, but usually consisted of a mixture of bread and milk, table scraps and varying amounts of meat.

Experimental observations. These observations were made on three bile fistula dogs of very different type and are representative of the reaction of normal dogs under these conditions. One dog (20-99) was a very active, young, white bull dog, nervous and excitable but raised in the laboratory kennels and undisturbed by the laboratory routine. This dog had a strong habit spasm of its shoulder muscles. Bile

Digitized by Google

was completely excluded from the duodenum as shown by autopsy. Another dog (18-123) was a young adult mongrel, active and lively but good natured and not disturbed by the laboratory routine. Bile was completely excluded from the duodenum as shown by autopsy. The third dog (18-30) was a very strong, vigorous and rather vicious female of about 6 years of age. She was in perfect physical condition during the 2 years of experimental work. Her appetite was capricious and she refused many food mixtures. At operation the bile fistula was made as usual but the common duct only ligated, not cut. In due time, a small communicating channel was reëstablished permitting the flow of a little bile into the duodenum when external drainage was not in effect (at night and outside of collection periods). Autopsy showed the condition as described and recognized clinically.

Descriptions of bile fistula dogs. Following are the records of the essential points in the life histories of the dogs used in this work; also certain post-mortem findings.

Dog 20-99. White bull, young male. Weight on March 26, 1920, 32.5 pounds. Dog has marked nervous tic of both fore limbs. Operation on March 26, 1920—common duct cut between ligatures; fistula made as usual. Recovery speedy and uneventful. Dog set up on April 5th for daily bile collections. Used in bile metabolism work from this time until January 29, 1921. Stools clay colored. January 26, 1921, blood serum quite icteric. Red cell hematocrit, 38 per cent. On January 31st, dog was observed to be very feeble and collections of bile were discontinued. Dog went rapidly downhill and on February 8th began to bleed from his fistula. Dog bled all night and in morning red cell hematocrit was 33 per cent, serum being decidedly icteric. Dog given excess of chloroform and immediately autopsied.

Autopsy findings: Remains of an emaciated dog (weight 20 lbs.). Fistula appears normal externally, but a bloody bile is exuding from it. Gums and other mucous membranes are extremely anemic. Median incision reveals normal subcutaneous tissues and muscles. There are a few fibrous adhesions between the omentum and operative site. The intestine and pancreas are stained a dark, maple sugar color. The costo-chondral junctures are enlarged. The ribs are very fragile and almost completely destitute of lime salts. Almost all the ribs show fusiform enlargements where spontaneous fractures with subsequent healing have occurred. The flat bones show the same process. The long bones of the extremities seem normal and contain normal marrow. The bones of the skull show some diminution of lime salts.

Heart and lungs—negative. Thyroid—small and pale. Spleen—negative.

Liver appears normal. No cirrhotic thickening. Slight scarring on a portion of ventral surface. Gall passages—hepatic and cystic ducts are patent and moderately dilated. Common duct is completely occluded and ends in a blind pouch.

Pancreas—deep, maple sugar color. Negative, on section. Duct opens normally into duodenum. Stomach and intestines—negative except for brown color

Digitized by Google

of muscle coats. Kidneys and bladder and prostate—negative. Adrenals—negative. Lymph nodes—a few are enlarged in region of liver hilus, also a few of the mesenteric nodes are enlarged. Brain appears negative superficially.

Microscopical sections: Liver—slight degree of round cell infiltration around some of smallest ducts. No cirrhosis. Moderate degree of brown atrophy. Central cells pale and contain brown pigment. A study of the bone abnormalities will be reserved for a future communication.

Dog 18-30. White bull, middle age, female. Weight on August 19, 1919, 39 pounds. Operation on August 19, 1919. Three ligatures placed on duct, but duct not cut. Fistula as usual. Recovery uneventful. Dog set up on September 10th. November, 1920, feces gave positive test for stercobilin with Schlesinger's reagent, indicating that exclusion of bile from duodenum is incomplete. Dog keeps her weight well. June 15, 1921, dog normal. Chloroform anesthesia and killed; autopsy performed at once.

Autopsy findings: Heart—normal. Lungs—slight anthracosis, normal on section. Spleen—rather large, firm and fibrous on section.

Liver—normal size, red-brown in color with occasional yellowish tinge; lobulation normal on section. Gall bladder attached to abdominal wall by way of operative fistula. A fine probe can be passed from papilla into common duct. Site of operation shows a definite stricture leaving an aperture not over 1 mm. in diameter. A slight dilatation exists above this. Probably drainage into duodenum was pretty efficient at night.

Stomach and intestines—show congestion due to a toxic proteose administered a few days previously. Pancreas—quite firm; section normal. Kidneys—fairly large in size, capsule adherent; section normal. Bladder—normal. Skeletal muscle—normal; slight amount of fat. Bones—apparently no thinning and no loss of lime salts. Bone marrow—no hyperplasia; normal in appearance.

Dog 18-123. White and tan, adult, female. Weight on October 9, 1919, 25 pounds. October 9th, exposure to x-rays over spleen region (200 milliampere minutes at distance of 10 inches from skin). Operation on October 21, 1919. Weight 26 pounds. Common duct cut and ligated above and below. Fistula as usual. Recovery uneventful. Dog set up on October 31st. Stools clay colored. Used for daily collections from this time until March 8, 1921, when she became intoxicated. Died March 10th.

Autopsy findings: Remains of an emaciated dog. Fistula externally appears in normal condition. Abdomen is moderately distended. Median incision reveals lack of subcutaneous fat. The ribs are quite fragile, containing a very small amount of lime salts. They bear numerous greyish enlargements, at the sites of previously healed fractures. The lower end of the sternum and the adjacent costal cartilages are bulged forward.

Heart and lungs—negative. Liver—a few small scars are present over the surface of the organ. It is negative on section. The larger ducts are much distended. Common duct is completely occluded at operative site. Spleen—small (12 × 3 × 0.5 cm.); weight—11 grams. On section, the fibrous elements are seen to be increased in amount. Kidneys—pale in color; capsules strip readily. Over the surfaces are a few small retention cysts. Pelves—negative. Adrenals—negative. Pancreas—brown in color; negative, on section. Intestines—brown color in muscle coats, moderate distention with gas.

Digitized by Google

Microscopical sections: Liver—central cells of lobules show beginning disintegration. Their nuclei show some degree of pyknosis, while cytoplasm is very pale and reticulated. Some of these cells contain masses of brown material. Peripheral cells of lobule—normal in appearance. No cirrhotic changes. No cholangitis. Spleen—capsule and trabeculae thickened. Malpighian bodies normal. Sinusoids widely patent and with red cells in them. Pulp cells appear normal. There is probably a slight relative decrease in parenchyma cells.

Table 1 gives three similar experimental periods on the same dog. This bile fistula animal had been under observation on the standard laboratory routine for about 13 months. The only complicating detail was an exposure of the splenic area to a considerable x-ray dose 2 weeks before the fistula operation. This dog shows the fluctuation in bile salts which are observed and so far are unexplained. We note the rise in bile salts following a change from a carbohydrate to a meat diet in the first and second experiments. This certainly is a physiological constant. The 24-hour collections show a moderately uniform series. There are fluctuations as are observed in the daily 6-hour periods but in general the four periods of 6 hours each in the 24-hour unit show uniform figures for volume and bile salts. We can detect no difference in this dog between the day and night collection periods. The bile pigment figures in this dog are high—in fact, more than twice normal and we observe some remarkable fluctuation in values. We cannot explain these reactions but they are more frequent in dogs with splenectomy as reported elsewhere (6).

Table 2 shows two experimental periods on the same dog. We note no difference during the night and day periods of the 24-hour collections. The general picture is very much like that noted in table 1.

Table 3 shows two more meat feeding experiments on the third dog of this series. The general type of reaction is the same as in the other two dogs. The rise in bile acids when the dog changes from a carbohydrate to a meat diet is very pronounced and probably more conspicuous in this dog which always ate sparingly of the carbohydrate mixtures but largely of the meat diet. During the second 24-hour period, the dog struggled very vigorously in an attempt to get out of the binder, but to no effect. There is a rise in bile volume flow but little other change to be attributed to this over-activity. As a rule during the night and much of the day, this dog dozed comfortably in its binder. There are very great fluctuations in bile pigment values.

Table 4 shows three collection periods on the same dog on a carbohydrate diet. There is a fairly uniform level of bile acid excretion

Digitized by Google

TABLE 1

Twenty-four hour bile collections after meat feeding

Dog 18–123. Brindle, mongrel, adult, female

DATE, 1920		VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
			In 1 cc. bile	Total in 6 hours				
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
9/27		26	0.266	6.92	254	50.6	18.0	Mixed diet
9/28		30	0.269	8.07	296	32.9	19.0	Carbohydrate diet
9/29		38	0.324	12.31	452	25.2	18.8	Carbohydrate diet
9/30		32	0.352	11.26	413	37.8	18.0	Beef scraps (430 gm.)
10/1	a	40	0.390	15.60	572	50.2	18.3	Beef scraps (1000 gm.) 10 a.m.–4 p.m.
	b	43	0.321	13.07	479	61.0		Dog vomited mucus 4 p.m.–10 p.m.
10/2	c	38	0.460	17.48	642	40.3		Dog vomited mucus 10 p.m.–4 a.m.
	d	45	0.348	15.66	574	51.7		4 a.m.–10 a.m.
11/15		29	0.112	3.25	119	37.9	17.5	Carbohydrate diet (1000 gm.)
11/16		22	0.180	3.96	145	25.3	17.5	Carbohydrate diet (500 gm.)
11/17		32	0.318	10.18	374	33.4	17.0	Meat diet (600 gm.)
11/18	a	49	0.443	21.71	797	32.8	17.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
	b	37	0.255	9.43	344	15.7		4 p.m.–10 p.m.
11/19	c	40	0.398	15.92	584	17.9		10 p.m.–4 a.m.
	d	21	0.417	18.76	688	98.6		4 a.m.–10 a.m.
11/29		35	0.439	15.36	564	42.4	18.5	Carbohydrate diet (870 gm.)
11/30		28	0.595	16.66	611	29.7	17.5	Carbohydrate diet (700 gm.)
12/1		27	0.308	8.31	303	30.6	17.0	Meat diet (350 gm.)
12/2		32	0.397	12.70	466	54.7	16.0	Meat diet (600 gm.)
12/3	a	45	0.496	22.32	818	45.4	16.5	Meat diet (425 gm.) 10 a.m.–4 p.m.
	b	45	0.368	16.56	607	39.5		4 p.m.–10 p.m.
12/4	c							10 p.m.–4 a.m.
	d	27	0.410	11.07	406	45.1		4 a.m.–10 a.m.

Digitized by Google

during most of the collection periods. In two of the observation periods there is a fall in bile salt output in the early morning hours. This may or may not be associated with a fall in volume output. The bile pigment output is very irregular and periods of semi-obstruction in the bile passages may have been responsible.

TABLE 2

Twenty-four hour bile collections after meat feeding

Dog 20-99. White bull dog, young, adult male

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/29	37	0.411	15.21	558	56.7	28.0	Carbohydrate diet (975 gm.)
11/30	46	0.170	7.82	286	54.0	26.0	Carbohydrate diet (700 gm.)
12/1	35	0.280	9.80	358	34.1	25.5	Meat diet (800 gm.)
12/2	50	0.326	16.30	598	22.1	25.0	Meat diet (600 gm.)
12/3 a	59	0.354	20.89	766	36.3	24.8	Meat diet (450 gm.) 10 a.m.-4 p.m.
12/3 b	50	0.283	14.15	519	47.0		4 p.m.-10 p.m.
12/4 c	48	0.198	9.50	349	63.0		10 p.m.-7 a.m.
12/4 d	40	0.382	15.28	561	33.4		7 a.m.-1 p.m.
11/15	30	0.267	8.01	292	35.5	26.0	Carbohydrate diet (1000 gm.)
11/16	51	0.194	9.89	361	65.0	26.0	Carbohydrate diet (500 gm.)
11/17	53	0.165	8.74	319	25.8	25.8	Meat diet (600 gm.)
11/18							Meat diet
11/19 a	25	0.318	7.95	290		26.0	Meat diet (475 gm.) 10 a.m.-4 p.m.
11/19 b	21	0.141	2.96	108			4 p.m.-10 p.m.
11/20 c	38	0.142	5.40	197			10 p.m.-4 a.m.
11/20 d	36	0.172	6.20	226			4 a.m.-10 a.m.

Table 5 shows a series of three observations on another dog receiving a carbohydrate diet. This dog shows a fairly uniform output of bile pigment. The bile salt output is not decreased during the night hours.

Table 6 is of considerable interest and shows a dog with incomplete bile fistula on a carbohydrate diet. At night, when this dog's fistula was closed, there could flow into the duodenum a certain amount of bile. This bile influx increased the bile acid flow of the following morn-

Digitized by Google

ing. This fact, established at autopsy, we believe explains at least in part the fall in bile salts noted in both 24-hour periods. This fall in bile salts is progressive after the first 6-hour collection and reaches its lowest level in the fourth collection period in the 24-hour experiment. The reaction therefore is much like that following an ingestion of bile in the early morning (refer to table 7). The bile pigment output is

TABLE 3

Twenty-four hour bile collections after meat feeding

Dog 18–30. Bull dog, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile.	Total in 6 hours.				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/15	15	0.448	6.72	247	87.7	34.0	Carbohydrate diet (170 gm.)
11/16	11	0.638	7.02	258	62.0	33.5	Carbohydrate diet (225 gm.)
11/17	17	0.971	16.51	606	85.3	33.5	Meat diet (600 gm.)
11/18 a	26	0.830	21.58	792	99.1	34.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
b	9	0.770	6.93	254	10.7		4 p.m.–10 p.m.
11/19 c	11	0.908	9.99	367	9.2		10 p.m.–4 a.m.
d	17	0.948	16.12	592	23.3		4 a.m.–10 a.m.
11/29	9	1.500	13.50	495	72.4	36.0	Carbohydrate diet (450 gm.)
11/30	8	1.120	8.95	329	102.0	34.5	Carbohydrate diet (450 gm.)
12/1	16	0.644	10.30	378	116.8	35.0	Meat diet (800 gm.)
12/2	30	0.738	22.14	810	41.8	35.0	Meat diet (600 gm.)
12/3 a	15	0.850	12.75	468	43.3	35.0	Meat diet (450 gm.) 10 a.m.–4 p.m.
b	32	0.549	17.57	644	21.2		Dog struggling 4 p.m.–10 p.m.
12/4 c	22	0.595	13.09	480	16.9		10 p.m.–4 a.m.
d	17	0.877	14.91	547	111.3		4 a.m.–10 a.m.

high but pretty uniform. The volume output in this dog is constantly subnormal as compared with the average animal.

Table 7 gives the results of three experiments on different bile fistula dogs, given large doses of taurocholic acid (2 grams) in solution by stomach. The reaction is remarkably uniform in these three different animals. The great cholagogue action does not modify the bile pigment

Digitized by Google

TABLE 4

Twenty-four hour bile collections after carbohydrate feeding

Dog 20-99. White bull dog, adult, male

DATE, 1920		VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
			In 1 cc. bile	Total in 6 hours				
		cc.	mgm.	mgm.	mgm.	mgm.	lbs.	
10/11		36	0.253	9.11	333	25.1	27.0	Mixed diet
10/12		44	0.283	12.45	457	21.4	26.3	Mixed diet
10/13		11	0.224	2.46	90	2.9	26.8	Carbohydrate diet (650 gm.)
10/14		63	0.112	7.05	258	20.5	25.5	Carbohydrate diet (1000 gm.)
10/15	a	23	0.140	3.22	118	15.1	25.0	Carbohydrate diet (1000 gm.) 10 a.m.-4 p.m.
	b	54	0.211	11.39	418	30.1		4 p.m.-10 p.m.
10/16	c	17	0.407	6.92	253	21.3		10 p.m.-4 a.m.
	d	21	0.309	6.49	237	31.4		4 a.m.-10 a.m.
11/8		47	0.438	20.58	755	58.9	27.8	Mixed diet (1150 gm.)
11/9		57	0.397	22.62	830	117.0	27.5	Carbohydrate diet (1000 gm.)
11/10		45	0.295	13.27	487	88.5	27.3	Carbohydrate diet (1000 gm.)
11/11		49	0.337	16.50	605	112.7		Carbohydrate diet (1000 gm.)
11/12	a	38	0.308	11.70	429	83.2	26.3	Carbohydrate diet (700 gm.) 10 a.m.-4 p.m.
	b	30	0.322	9.66	353	61.6		4 p.m.-10 p.m.
11/13	c	36	0.280	10.08	370	72.7		10 p.m.-4 a.m.
	d	27	0.364	9.82	355	72.4		4 a.m.-10 a.m.
12/6		55	0.255	14.02	514	15.3	24.5	Carbohydrate diet (1000 gm.)
12/7		40	0.312	12.48	457	62.4	24.5	Carbohydrate diet (700 gm.)
12/8		40	0.326	13.04	478	89.8	24.0	Carbohydrate diet (1000 gm.)
12/9		41	0.252	10.33	379	57.0	23.5	Carbohydrate diet (1000 gm.)
12/10	a	38	0.227	8.62	314	50.8	23.5	Carbohydrate diet (1000 gm.) 10 a.m.-4 p.m.
	b	27	0.241	6.51	238	38.8		4 p.m.-10 p.m.
12/11	c	37	0.113	4.18	153	40.3		10 p.m.-4 a.m.
	d	28	0.284	7.95	290	53.0		4 a.m.-10 a.m.

Digitized by Google

TABLE 5

Twenty-four hour bile collections after carbohydrate feeding

Dog 18-123. Brindle mongrel, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	mgm.	mgm.	mgm.	mgm.	lbs.	
10/11	31	0.352	10.91	400	41.4	18.0	Mixed diet
10/12	26	0.297	7.72	282	22.6	17.0	Mixed diet
10/13	48	0.322	15.45	568	20.9	18.0	Carbohydrate diet (620 gm.)
10/14	32	0.154	4.93	180	24.5	17.0	Carbohydrate diet (830 gm.)
10/15 a	18	0.267	4.81	176	27.2	16.5	Carbohydrate diet (710 gm.) 10 a.m.-4 p.m.
10/15 b	40	0.168	6.72	245	27.8		4 p.m.-10 p.m.
10/16 c	14	0.225	3.15	115	15.3		10 p.m.-4 a.m.
10/16 d	5				6.1		4 a.m.-10 a.m.
11/8	29	0.297	8.61	314	47.4	17.0	Mixed diet
11/9	23	0.340	7.82	286	36.0	17.0	Carbohydrate diet (750 gm.)
11/10	30	0.295	8.86	324	33.7	17.5	Carbohydrate diet (1000 gm.)
11/11	38	0.309	11.75	432	32.2		Carbohydrate diet (860 gm.)
11/12 a	36	0.280	10.08	370	34.3	17.5	Carbohydrate diet (650 gm.) 10 a.m.-4 p.m.
11/12 b							4 p.m.-10 p.m.
11/13 c	23	0.322	7.41	271	50.0		10 p.m.-4 a.m.
11/13 d	22	0.392	8.62	351	53.6		4 a.m.-10 a.m.
12/6	36	0.255	9.18	337	117.9	16.3	Carbohydrate diet (800 gm.)
12/7	25	0.071	1.77	65	37.0	16.5	Carbohydrate diet (675 gm.)
12/8	23	0.298	6.85	251	36.0	16.5	Carbohydrate diet (730 gm.)
12/9	26	0.098	2.54	93	36.2	16.5	Carbohydrate diet (800 gm.)
12/10 a	24	0.156	3.74	137	25.5	16.5	Carbohydrate diet (1000 gm.) 10 a.m.-4 p.m.
12/10 b	36	0.156	5.61	206	35.1		4 p.m.-10 p.m.
12/11 c	21	0.129	2.81	103	22.3		10 p.m.-4 a.m.
12/11 d							4 a.m.-10 a.m.

Digitized by Google

output. The greater part of the 2 grams of taurocholic acid is eliminated in the 6 hours following its ingestion. In spite of this, the cholagogue action is much in evidence in the second period of 6 hours. This period of cholagogue action without any excess of bile salt excretion shows that the outpouring of an excess of fluid in bile is not a simple reaction to preserve a certain salt concentration in the bile passages.

TABLE 6

Twenty-four hour bile collections after carbohydrate feeding

Dog 18-30. Bull dog, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/8	6	1.170	7.02	257	51.2	35.8	Mixed diet (800 gm.)
11/9	8	0.964	7.71	283	69.6	35.0	Carbohydrate diet (70 gm.)
11/10	4	1.475	5.90	216		34.5	Carbohydrate diet (210 gm.)
11/11	10	0.857	8.57	314	100.0	34.0	Carbohydrate diet (230 gm.)
11/12 a	9	1.025	9.22	338	98.3	33.5	Carbohydrate diet (220 gm.) 10 a.m.-4 p.m.
11/12 b	7	0.616	4.31	158	85.1		4 p.m.-10 p.m.
11/13 c	10	0.364	3.64	134	96.0		10 p.m.-4 a.m.
11/13 d	6	0.420	2.52	92	71.9		4 a.m.-10 a.m.
12/6						35.0	Carbohydrate diet (50 gm.)
12/7	8	1.690	13.52	496	84.6	34.5	Carbohydrate diet (200 gm.)
12/8	10	1.090	10.90	400	98.3	34.0	Carbohydrate diet (370 gm.)
12/9	11	0.882	9.70	356	110.3	33.5	Carbohydrate diet (475 gm.)
12/10 a	22	0.383	8.43	309	51.3		Carbohydrate diet (490 gm.) 10 a.m.-4 p.m.
12/10 b	17	0.412	7.00	257	76.2		4 p.m.-10 p.m.
12/11 c	5	0.925	4.62	169	88.4		10 p.m.-4 a.m.
12/11 d	5	0.570	2.85	105	146.0		4 a.m.-10 a.m.

This emphasizes the ease with which the various elements of bile secretion may be dissociated—the fluid and salt elements and bile pigments. This all speaks for a remarkable diversity in functional activity of the liver cell. It may be well to note that this crude solution of taurocholic acid was obtained from whole dog's bile evaporated with charcoal to a soft paste, extracted with water and filtered. Obviously other elements besides taurocholic acid are present.

Digitized by Google

TABLE 7

Twenty-four hour bile collections after bile feeding

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
Dog 18–123. Brindle, mongrel, adult, female							
	cc.	mgm.	mgm.	mgm.	mgm.	lbs.	
12/13	38	0.410	15.58	572	40.7	16.5	Mixed diet
12/14	38	0.314	11.93	440	33.3	16.5	Meat diet
12/15	40	0.422	16.88	620	34.8	17.0	Meat diet
12/16	58	0.434	25.17	924	33.7	17.0	Mixed diet
12/17 a	27	0.412	11.12	408	26.9	16.8	Mixed diet 10 a.m.–4 p.m.
12/17 b	70	0.909	63.63	2335	18.3		*4 p.m.-10 p.m.
12/18 c	36	0.369	13.88	509	22.7		10 p.m.–4 a.m.
12/18 d	18	0.468	8.42	309	24.7		4 a.m.–10 a.m.
Dog 18–30. Bull dog, adult, female							
12/13	13	0.452	5.87	215	61.5	33.5	Mixed diet
12/14	16	0.814	13.02	478	80.2	34.0	Mixed diet
12/15	13	1.100	14.30	525		34.0	Mixed diet
12/16	14	1.428	19.99	733	61.5	33.5	Mixed diet
12/17 a	7	0.800	5.60	206	43.9	33.3	Mixed diet 10 a.m.–4 p.m.
12/17 b	60	1.250	75.00	2755	67.2		*4 p.m.–10 p.m.
12/18 c	11	1.010	11.11	408	46.0		10 p.m.–4 a.m.
12/18 d	8	1.190	9.52	349	52.3		4 a.m.–10 a.m.
Dog 20–99 White bull dog, young adult, male							
12/13	40	0.254	10.16	373	33.4	23.5	Mixed diet
12/14	48	0.285	13.68	502	28.4	24.0	Mixed diet
12/15	50	0.183	9.15	334	25.0	24.0	Mixed diet
12/16	50	0.238	11.90	437	41.8	23.5	Mixed diet
12/17 a	32	0.337	10.78	396	41.8	23.3	Mixed diet 10 a.m.–4 p.m.
12/17 b	67	0.955	63.98	2347	36.5		*4 p.m.–10 p.m.
12/18 c	63	0.211	13.29	487	35.6		10 p.m.–4 a.m.
12/18 d	45	0.211	9.45	345	32.9		4 a.m.–10 a.m.

* At 4 p.m., a solution of extracted dog's bile, containing 2 grams of taurocholic acid, made up to 400 cc. with water, was given by stomach tube.

Digitized by Google

DISCUSSION

In all experiments with bile fistula dogs we believe it essential to work with animals of two types—one with complete exclusion of bile from the duodenum and one with incomplete exclusion or a little seepage of bile through a small strictured common duct. This second type is well illustrated above (dog 18-30) and is of much importance. These dogs during drainage periods will certainly yield a complete bile flow as the flow through the stricture is not easy and in the standing posture the escape of bile into the cannula is very prompt and complete. But during resting or night periods there is a flow of an undetermined volume of bile into the duodenum and this is sufficient to maintain these dogs in perfect physical condition. There are certain objections to both types of experimental animal but a combination of these types gives data which we believe are trustworthy and of real physiological significance.

Dogs with biliary fistulas and complete exclusion of bile from the duodenum suffer from a variety of peculiar intoxications and metabolic disturbances, some of which have been noted previously. We record certain bony changes in the autopsy notes of two of the bile fistula dogs. (Dogs 20-99 and 18-123.) These abnormalities are of considerable interest as relating to the metabolism of inorganic salts and will be the subject of a future communication.

Our experiments with the long 24-hour collections give no constant results to indicate a decided fall in output at night or during the early morning hours. The amounts of bile acids and bile pigments are not uniformly decreased during the periods of sleep. The same statement applies to volume output of bile.

We wish again to point out the ease with which one can dissociate the various constituents of the bile (table 7). Large doses of crude taurocholic acid cause a prompt rise in bile salt excretion so that practically all the taurocholic acid is excreted within 6 hours after its ingestion. But the cholagogue action persists during the second 6-hour period or longer, showing dissociation of salt and fluid elimination. The bile pigment output remains constant throughout.

One can detect no difference in cholagogue action whether the bile salt is given during the morning or evening. The reaction will be the same during the day or during night periods when the dog is sleeping comfortably most of the time.

Digitized by Google

SUMMARY

Bile fistula dogs will show little if any difference in the output of bile, bile salts or bile pigments during four consecutive 6-hour periods. There are no constant differences to be made out. If anything can be said, it is to the effect that at times the night output is slightly less than the normal daily output. This applies to healthy bile fistula dogs.

Peculiar bony abnormalities are noted as related to the constant loss of bile in such animals.

Complete dissociation of the bile constituents can be demonstrated (table 7) after a large dose of taurocholic acid. Following a 2-gram dose we observe a great rise in bile salt content during the first 6-hour period. During the second 6-hour period the bile salt content is about normal but the cholagogue action is still much in evidence and the fluid output therefore is dissociated from the bile salts. The bile pigments are constant before and after the period of bile salt administration and elimination.

This all speaks for a remarkable diversity in liver cell function—a point not too frequently emphasized.

BIBLIOGRAPHY

(1) WHIPPLE AND HOOPER: This Journal, 1916, xl, 349.
(2) WHIPPLE AND HOOPER: This Journal, 1917, xlii, 256.
(3) FOSTER, HOOPER AND WHIPPLE: Journ. Biol. Chem., 1919, xxxviii, 393.
(4) HOOPER AND WHIPPLE: This Journal, 1916, xl, 332.
(5) FOSTER AND HOOPER: Journ. Biol. Chem., 1919, xxxviii, 355.
(6) HOOPER AND WHIPPLE: This Journal, 1917, xliii, 275.

Digitized by Google

THE RELATIONSHIP BETWEEN NERVOUS AND HORMONE CONTROL OF THE RESPIRATORY CENTER

J. J. R. MACLEOD AND S. U. PAGE

From the Department of Physiology, University of Toronto, Toronto, Canada

Received for publication November 23, 1921

The changes which can be brought about in the activity of the respiratory center in laboratory animals by various forms of sensory stimulation and by alterations in the composition of the blood are often decidedly variable and this is the case not only for animals of different species but also, not infrequently, for individuals of the same species. There can be no doubt that a part of this inconstancy in results is dependent on the fact that the observations have usually been made on animals that are under varying degrees of anesthesia. The degree of anesthesia affects the respiratory center in part because this is directly depressed by the anesthetic and, in part, because of dulling of the sensitivity of the higher (affective) centers which, it is well known, have a close relationship in the conscious animal with changes in respiration. Thus, in a conscious animal even the mildest stimulation of the pain receptors is usually associated with a decided change in breathing, which is not always the case under conditions of general anesthesia.

In order to eliminate the errors due to unequal degrees of anesthesia we have chosen, for investigation of the behavior of the respiratory center, animals in which the higher centers have been removed by section of the brain stem about the level of the anterior corpora quadrigemina. In certain animals, notably the cat, this operation usually furnishes a preparation in which the breathing proceeds with more or less regularity for many hours and, if the decerebration be quickly performed immediately after placing the animal deeply under ether, every trace of the anesthetic is removed from the blood within an hour after the decerebration. As has been pointed out elsewhere (1) many cats do not respire normally after the decerebration, a condition of hyperpnea becoming gradually developed for the incidence of which the exact position of the section of the brain stem and the age of the animal are the chief determining factors.

Digitized by Google

In a successful decerebrate preparation the chief respiratory center is isolated from two types of impulses which in the intact animal greatly influence its activity, namely, those from the higher (cerebral) centers and those from the nasal mucosa. Although this partial isolation must be borne in mind in the interpretation of the results obtained by experimental alterations in the activity of the center, many of these are of so definite and constant a nature that they must be considered as representing conditions which come into play in the intact animal.

The observations recorded in the present paper have been made on animals (mainly cats) in which the breathing was perfectly regular one hour after decerebration by Sherrington's method. To prevent fall in body temperature the preparations were kept on a heated table with the head end somewhat raised on a hot water bag. The rectal temperature was frequently observed and the heating suitably adjusted if any changes in this were observed to occur.

Several related problems have been investigated all bearing on the general question of the excitability of the center during alterations in the composition of the blood, or after removal of the afferent impulses arriving at it through the vagus nerves. The results are grouped under various headings.

THE EFFECT PRODUCED ON BREATHING BY SECTION OF THE VAGUS NERVES. It is usually stated that the action of the respiratory center besides becoming very slow also becomes irregular and inadequate when the vagus nerves are cut in animals from which the higher centers have been removed (2). We have found this to be the case in decerebrate rabbits but not, as a rule, in decerebrate cats. Decerebration in animals of the former group is not generally so successful as in those of the latter and it is best performed by means of a blunt hook passed into the brain case through a trephine hole. Only a few of the decerebrations are really successful in the sense that regular and efficient breathing persists after the operation, but when it does so, section of both vagi invariably causes a complete breakdown and the rabbit soon dies of asphyxia. In some rabbits section of one vagus nerve is sufficient to have this effect, as is illustrated in the tracing of figure 1. In this case section of the right vagus was immediately followed by a prolonged period of apnea succeeded by very slow deep respirations. At the points indicated on the tracing small amounts of an approximately normal solution of hydrochloric acid were injected intravenously with the result that the breathing became deeper without any change in rhythm.

Digitized by Google

In decerebrate *cats* section of one vagus does not usually have any effect on breathing but section of the nerve on both sides causes the rhythm to decline and the depth to become greater. In the experiment shown in figure 2, the upper tracing is that from a tambour connected with the thorax and the lower, from a Gad-Krogh spirometer placed in series in a closed system of wide-bore tubing furnished with valves and soda-lime absorbers and connected with the trachea. The volume of air breathed in a unit of time is therefore determined by multiplying the height of each respiration by the rate. The air in the closed system

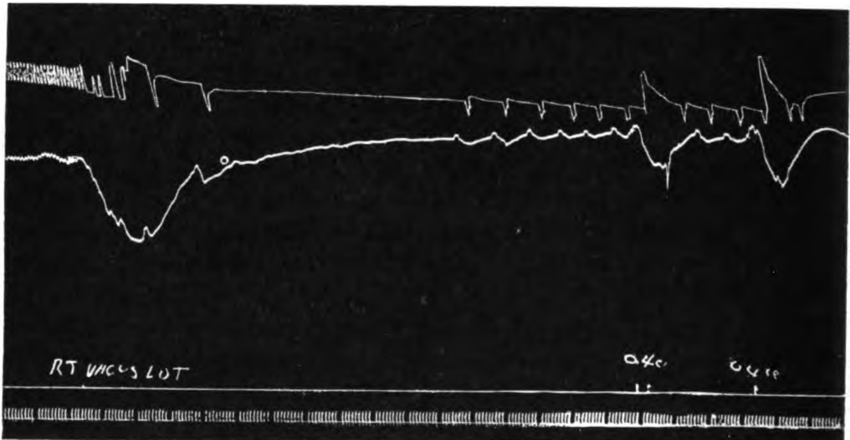


Fig. 1. (Rabbit 4) Cutting of vagus nerve on right side in decerebrated rabbit caused entire break down of respiratory rhythm (upper tracing) and temporary fall in blood pressure (lower tracing). The respirations were recorded by a balloon placed between the liver and diaphragm. The effect of injection into the peripheral end of one of the carotid arteries of 0.4 cc. of weak HCL in 0.9 NaCl is also shown. Time in seconds.

contained excess of oxygen. By comparison of the volume of air breathed per minute it will be observed that a decrease occurred immediately after vagotomy as in the experiment shown, where it decreased from 960 cc. to 700 cc. per minute. In such cases pulmonary ventilation is adequately performed with a lesser minute volume of air because each breath is deeper and therefore more thoroughly renews the air in the alveoli. The difference in minute volume is however not much changed as a rule and sometimes it may even be slightly increased and it is interesting that when progressively greater percentages of CO_2 are inspired it increases at about the same rate before as after vagotomy

Digitized by Google

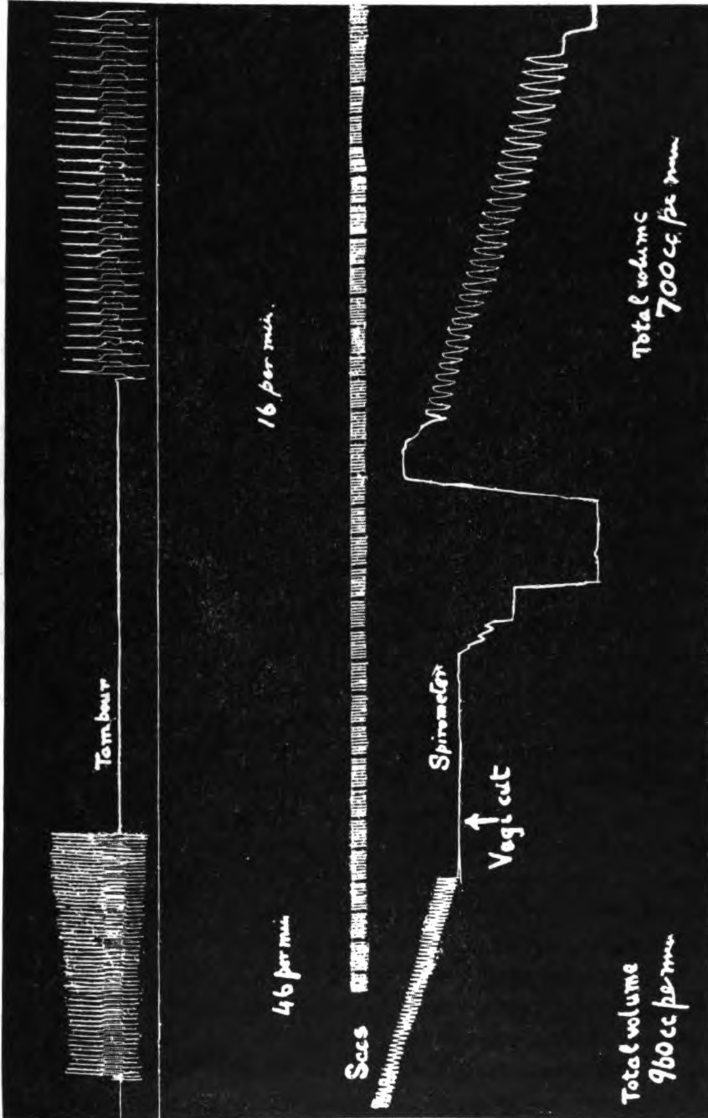


Fig. 2. (Cat 29) Cutting of both vagi in decerebrated cat caused the respirations to change from 45 to 15 and the volume from 960 cc. to 700 cc. per minute. Upper tracing tambour, lower, Krogh spirometer. Both before and after vagotomy, the breathing progressively increased during the periods of registration. Time in seconds.

Digitized by Google

(curve I, fig. 4). There were no signs of asphyxia in these animals after vagotomy—as judged from the behavior of the blood pressure, the longevity of the animal and the arterial character of the blood—and the oxygen consumption remains normal—as judged from the slope of the descent of the spirometer curve.

It is of interest to compare the rate of breathing before and after the vagotomy, as is done in the following table:

Rate of breathing

NUMBER	BEFORE VAGOTOMY	AFTER VAGOTOMY	RATIO
	per minute	*per minute*	
II	46	16	2.87:1
III (fig. 3)	41	20	2.05:1
Ia	42	14	3.00:1
IIIa	23	11	2.09:1

The fact comes to light that there is often a simple ratio between the rate before and after vagotomy. What this may mean is difficult to say. It suggests that there is a certain fundamental rhythm of the center which is independent of afferent vagal impluses and in the decerebrate animal at least is influenced mainly by the temperature of the blood and perhaps by extreme anoxemia and by poisons. It will be observed that this rhythm varied between 11 and 20 in our experiments, these differences being probably partly dependent on differences in temperature of the animal. Although this was observed regularly so as to indicate what degree of heat we should turn on to the table, it might vary by 1°C. and it is altogether likely that the temperature of the blood in the medulla would vary still more. This fundamental rhythm is accelerated 2 or 3 times by the vagal impulses but not apparently, in decerebrate animals, to intermediate degrees. Of course such ratios can be expected only when there has been no stimulation, of the respirations—either hormone or nervous—before the vagi were cut. If this operation be performed during CO_2-hyperpnea, for example, then, as shown in figure 3, no simple ratio is likely between the breathing immediately before and after.

It should be added that section of the vagi sometimes causes complete breakdown of breathing in decerebrate cats, but in our experience this is the exception rather than the rule. The exact position of the cut across the brain stem is the most important factor in determining whether or not vagotomy will cause respiratory failure. In our experi-

Digitized by Google

ence, if the anterior corpora quadrigemina be intact respiratory failure does not follow vagotomy, but if the cut involve these structures to any considerable extent it is likely to occur.

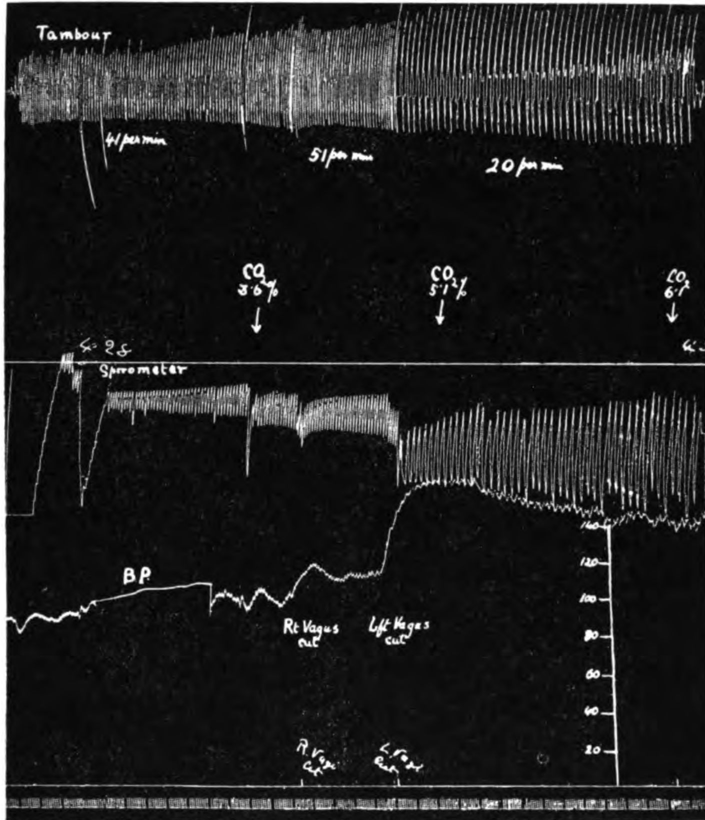


Fig. 3. (Cat 32) Cutting of both vagi in decerebrated cat caused the respirations to become slower but not irregular. At the time when the nerves were cut the animal was respiring in a closed system so that the percentage of CO_2 in the alveolar air was steadily rising, as indicated by the figures. Upper tracing, tambour; middle tracing, spirometer; lower tracing, arterial blood pressure. Time in seconds.

THE RELATIONSHIP BETWEEN THE HORMONE AND NERVOUS CONTROL OF THE RESPIRATORY CENTER. Current opinion is decidedly vague as to the precise relationship existing between hormone and afferent stimulation of the respiratory center. According to the usually accepted view

Digitized by Google

the rhythmic discharges of the center are regulated to a rate and depth that is adequate for efficient respiration, partly by the action of the respiratory hormones affecting the depth and partly by certain afferent stimuli (affecting mainly the rate) arriving at it principally by way of the vagus nerves. Afferent stimuli from other sources, and cerebral impulses, only occasionally act on the center, either exciting or inhibiting its rhythm temporarily. The question therefore arises as to whether maintained changes in either the nervous or the hormone stimulus may alter the excitability of the center toward occasional changes of the other form of stimulus.

a. The effect of vagotomy on the excitability of the center toward hormones. To determine whether the excitability of the center toward hormone stimulation becomes altered when it is almost entirely isolated from afferent stimuli, we have measured the effects produced on the minute volume of air breathed when the percentage of carbon dioxide in the inspired air is increased in decerebrate cats (by the same technique as that used by R. W. Scott (3), before and after vagotomy. The results of one of several experiments, all yielding corresponding results, are shown in the accompanying curves (fig. 4) in which the figures on the abscissa give the percentages of CO_2 in the inspired air, and those on the ordinates the number of cubic centimeters of air breathed per minute. The thick continuous line is drawn from the results obtained before vagotomy and the thick broken line from those after vagotomy in the same cat. The number of respirations is also depicted in the curves drawn in thin lines. It will be seen that there is a very close correspondence in the results of the two experiments. In no. I the initial respiratory volume is somewhat greater after decerebration than before (about 40 cc.) but by the time 2.5 per cent of carbon dioxide was being inspired the volumes have come to be the same. The rate of breathing (thin dotted line) increases along with the minute volume before vagotomy but remains unchanged after this operation until higher percentages of CO_2 are being inspired, when it may decline. During both experiments the rate of increase of breathing for different percentages of CO_2 is somewhat greater than the average given by R. W. Scott probably because a smaller volume of air was contained in the rebreathing system of tubing. This air was mixed with a large excess of oxygen, there being always well over 20 per cent of this gas present at the end of each observation. The parallelism in the curves persists until about 4 per cent of CO_2 was being breathed when the curve after vagotomy becomes less steep and at about 5 per cent actually begins to decline.

Digitized by Google

This failure of the respiratory response at higher percentages of CO_2 has not been invariably observed in other experiments of the same type.

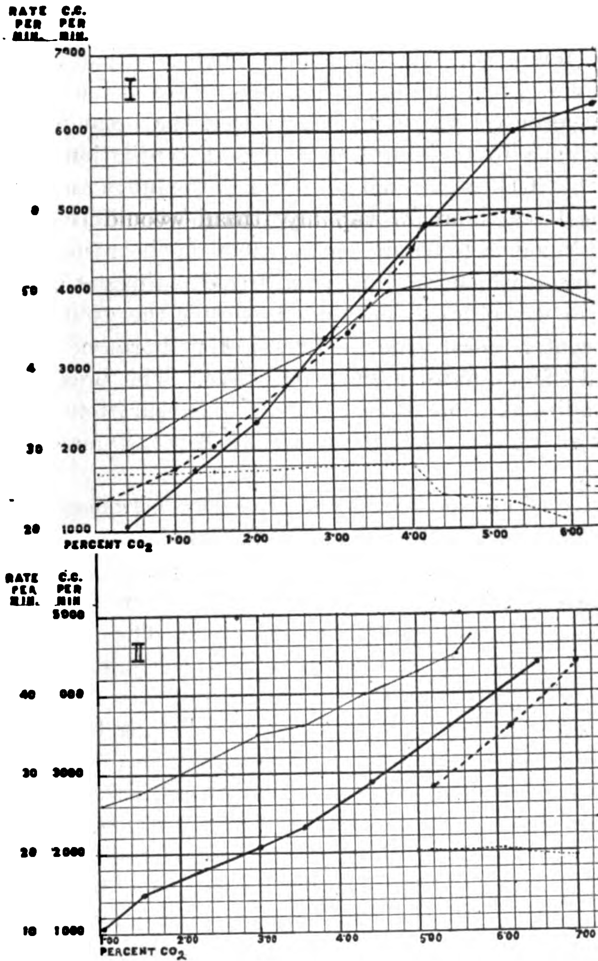


Fig. 4. Curves showing the relationship between breathing and progressively increased percentage of CO_2 in inspired air before and after vagotomy in decerebrate cats. Thick continuous and broken lines represent the minute volume of respired air, thin lines the rate of breathing per minute.

Thus it did not occur in the second series of curves shown in figure 4. When it occurs it is accompanied by a slowing of the breathing and it indicates that the limit of hyperpnea has been reached. This limit

Digitized by Google

might be determined either by the extent of expansion and collapse of the thorax or by exhaustion of the respiratory center. Examination of tracing of the respiratory movements does not throw light on this question because, being accelerated as well as deepened, each respiratory movement before vagotomy is of course much less than after it. The actual slowing of breathing that often supervenes at high CO_2 percentages after vagotomy leads us to believe that the breakdown is due in the first instance to the limit of increased ventilation having been reached so that CO_2 is less well got rid of in the lungs and by accumulating in the blood more rapidly than would be the case with freer breathing, develops a toxic anesthetic effect on the respiratory center.

The conclusion which we draw from the experiments is that section of the vagi in decerebrate cats does not alter the excitability of the respiratory center toward the chief respiratory hormone (CO_2-tension) at least until the stimulus becomes excessive. The breakdown under the latter conditions is due to the failure of the respirations to accelerate.

These observations confirm those of F. H. Scott (4) made on anesthetised rabbits.

b. The effect of alterations in the respiratory hormones on the reflex excitability of the center. In the following experiments the response of the respiratory center toward afferent nerve stimulation after intravenous injections of acid or alkali, or during breathing air that was rich in CO_2 or deficient in oxygen, was investigated. The central end of the sciatic or vagus nerve, placed in Sherrington's electrodes, was stimulated with the Faradic current and, the strength of stimulus just necessary to produce a distinct and constant response having been ascertained, the hormone stimulus was caused to vary, by the methods just mentioned, and the electrical stimulus again applied.

1. The effect of injections of sodium carbonate solution. It has already been pointed out that the respiratory effects which follow such injections are much less pronounced than was formerly believed to be the case (3). Only when comparatively strong solutions of this alkali are injected rapidly is there any decided diminution of breathing and R. W. Scott has shown that enough may be injected slowly to raise pH of the blood to 7.7 or 7.8 without much diminution in the minute volume of air breathed.

In figure 5 are shown the effects of stimulation of the sciatic nerve before and after the injection, during a period of 8 minutes, of 10 cc. of a 4 per cent solution of sodium carbonate in gum saline. According to Scott's results this should cause the blood to become decidedly alka-

Digitized by Google

line (3). With the secondary coil at 26 cm. before the injection the respirations were slowed, expiration being deepened, and there was a slight pressor effect on blood pressure. Immediately after the injection the results were as nearly as possible the same, being, if anything, slightly more pronounced. This experiment has been repeated several times and we could never convince ourselves that the alkaline injections

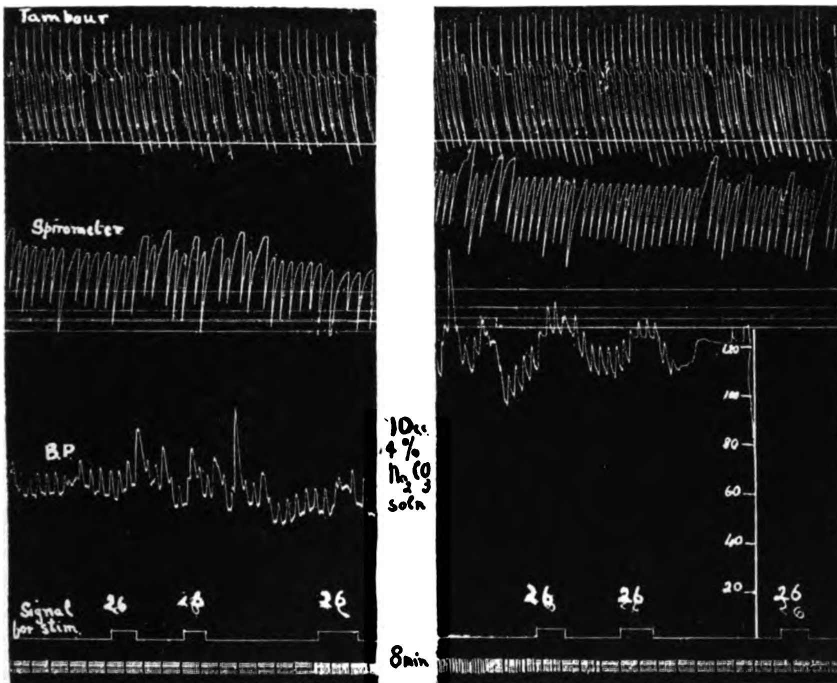


Fig. 5. (Cat 21, 2.5 kgm.) The effect of stimulation of the central end of the sciatic nerve before and after the injection of gum saline containing 4 per cent sodium carbonate. There is no evidence of depression in the excitability of the center although pH was presumably raised. Time in seconds.

had any constant effect one way or the other on the excitability of the center to nerve stimulation.

2. The effect of injections of acid solutions. These are shown in figure 6. With the secondary coil at 26 cm. inspiration was slightly excited and the blood pressure decidedly increased, and these results were not perceptibly altered immediately after the injections of as large amounts of a solution of lactic acid as the heart would tolerate. Even after

Digitized by Google

repeated injections of the acid solution, which were made in the interval elapsing between the two parts of the tracing, no increased excitability of the respiratory center could be demonstrated as is evident from the later part of the tracing.

3. The effect of breathing atmospheres rich in carbon dioxide. The objection may be raised to the above described experiments with acid on the gound that their effects on the H-ion concentration of the blood

Fig. 6. (Cat 21, 2.5 kgm.) Stimulation of central end of sciatic nerve before and immediately after the injection of N lactic acid in as large amounts as the animal would tolerate. Time in seconds.

supplying the respiratory center had passed off before the second series of afferent stimuli was applied. It became necessary to supply the acid continuously, but since we have found that continuous injections of solutions of mineral acids *are very liable to cause a sudden breakdown of the respiratory mechanism, at least in decerebrate cats,* we decided to bring about the desired increase in the hormone stimulus by causing the animals to breathe atmospheres containing sufficiently high percent-

Digitized by Google

ages of CO_2 definitely to excite the respirations. The effect of afferent nerve stimulation was then observed immediately before and during the hyperpnea.

The results of such an experiment are shown in figure 7. In this case the middle tracing (spirometer) was taken with a clamp applied to the outlet tube of the spirometer with a pressure which was sufficient to make the rate of filling and of emptying just balance for ordinary breathing. The very slightest increase or decrease in the minute volume is therefore indicated by a rise or fall in the level of the spirometer tracing. It will be observed that stimulation of the central end of one vagus nerve with the secondary coil at 45 cm. caused a slight increase in the minute volume which was practically the same during a prolonged state of hyperpnea produced by respiring CO_2-rich air as it was in the preceding and following periods. During one of the periods of stimulation (marked * on tracing) the administration of CO_2 caused a slightly greater response than the usual but this is the only instance in a considerable number of experiments in which such an effect has been observed.

Similar conclusions can be drawn from the tracing shown in figure 8 in the experiment of which the outlet tube of the spirometer was completely clamped at intervals so that its rate of filling might be determined. Unfortunately the capacity of the spirometer between the two lines is not exactly known for this experiment but this does not really matter for purposes of comparison, the rate of filling being proportional to the distances between the vertical lines. With the coil at 40 cm. stimulation of the central end of the sciatic nerve caused a marked rise in blood pressure and changed the rate of filling of the spirometer on an average from 17 seconds to 10 seconds and later from 15 seconds to 12 seconds, while the animal was breathing outside air. During the hyperpnea caused by breathing a CO_2-rich atmosphere the same strength of stimulus produced less effect on the blood pressure, the rate of filling of the spirometer being changed on an average from 14 seconds to 11 seconds and later, from 15 seconds to 12 seconds. There is in these results no evidence that the excitability of the center has been altered by carbon dioxide.

Although we have been unable to confirm Cohen (5), who performed similar experiments, we are prepared to admit that the problem demands still further investigation. Inasmuch as it has been shown, especially by Collip (6), that respiratory excitement may result from intravenous injection of bicarbonate solutions and in light of the observations of Jacobs (7) on the cause for the greater exciting effect

Digitized by Google

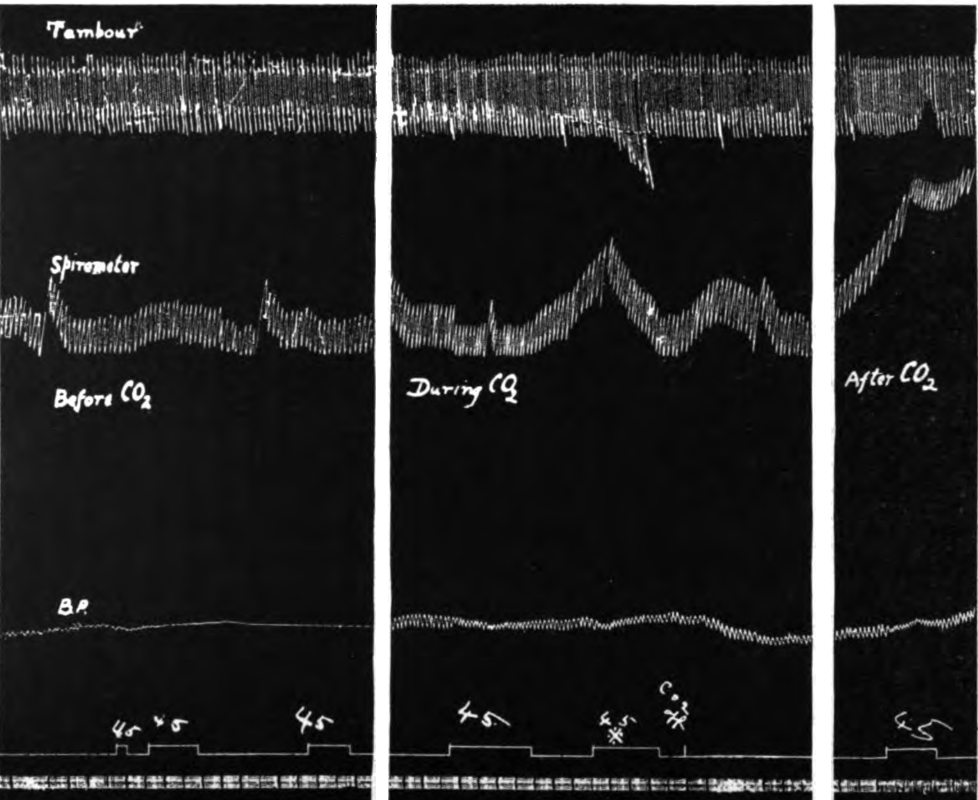


Fig. 7. (Cat 14) Stimulation of the central end of the vagus (at the periods indicated by signals) during and some time after breathing into an atmosphere containing an excess of CO_2. The respiratory tracing in this experiment was taken by connecting one tube of a Krogh spirometer with the expiration valve and partly clamping the other tube. A rise or fall in the level of the tracing, therefore, indicates increase or decrease in the volume of air breathed. The distance of secondary coil indicated by figures. Time in seconds.

Digitized by Google

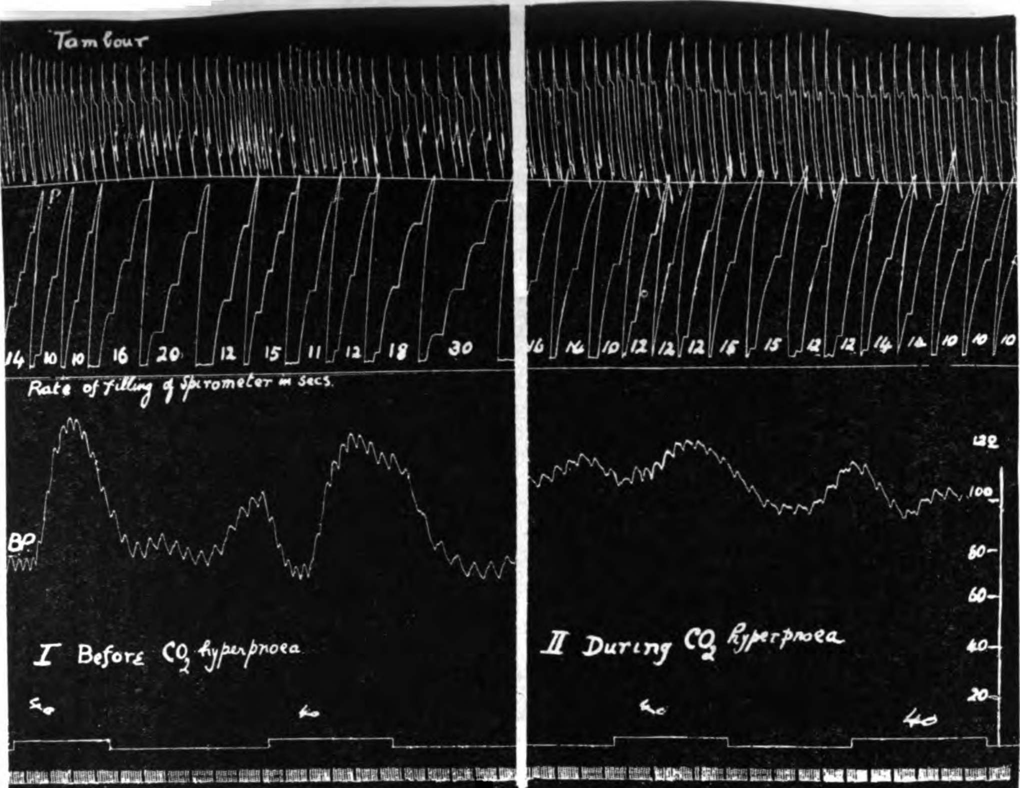


Fig. 8. (Cat 15) The effect of respiring CO_2-rich air on the excitability of the respiratory and vaso constrictor centers toward stimulation of an afferent nerve (sciatic). The uppermost tracing is that of a tambour connected with the trachea, the middle tracing shows the rate at which a Krogh spirometer filled to 240 cc., and the lowermost tracing is arterial blood pressure. The periods of stimulation are indicated by signals, the figures above which give the distance of the secondary from the primary coil. Tracing 1, breathing normal air; tracing 2, breathing CO_2-rich air. Time in seconds.

Digitized by Google

of CO_2 tension than would correspond to its effect on the CH of the blood, we propose to repeat this part of the investigation under more strictly controlled conditions of afferent nerve stimulation.

4. The effect of anoxemia. From the tracing shown in figure 9 it will be seen that the effect of stimulation of the central end of the vagus nerve

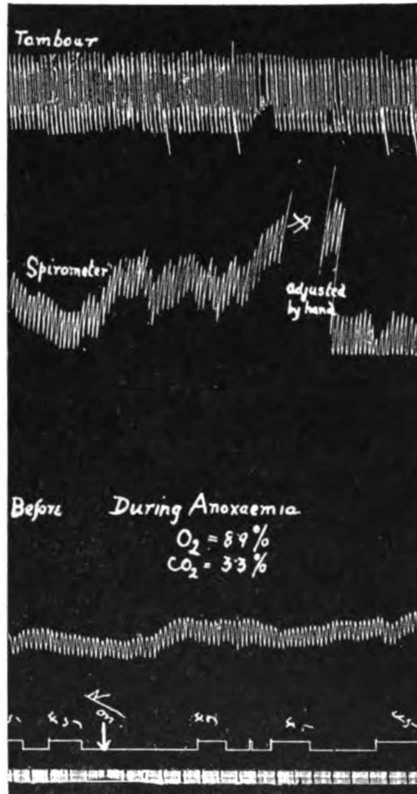


Fig. 9. (Cat 14) Stimulation of central end of vagus before and during breathing into an atmosphere very deficient in O_2. Time in seconds.

was the same when the animal was breathing in an atmosphere containing about 10 per cent O_2 as in outside air. The spirometer tracing was taken by the same method as in experiment 7. This observation has been repeated at various stages of anoxemia and always with the same result, i.e., at no stage in anoxemia can it be shown that the respiratory center is hyperexcitable to afferent nerve stimulation.

Digitized by Google

The influence of section of the vagus nerve on the gradual increase in breathing which supervenes shortly after causing an animal to breathe into a closed system of tubes. The occurrence of this form of hyperpnea has been noted in a previous communication (4) where also it is shown that deficiency of oxygen cannot be its cause. The slight resistance that is offered to the movement of air due to friction in the tubing, by raising the intrapulmonary pressure, was considered to be the cause of the hyperpnea although a similar increase of breathing could not be induced by weighting the spirometer. The only other possible explanation of the phenomenon is that the tension of CO_2 in the alveolar air becomes raised by breathing in the closed system (even although this contains CO_2 absorbers) and, consequently, the CO_2 tension in the blood. The more or less gradual onset of the hyperpnea would lend support to this view. As a matter of fact, however, the percentage of CO_2 in the alveolar air is decidedly decreased during the hyperpnea and R.Q. is raised (cf. 4). Nor can prolongation of the dead space be held accountable for the phenomenon.

If reflex stimulation of the respiratory center be the cause, it is presumably through the vagus nerves that the afferent impulses would pass. It is therefore of interest to observe the influence of section of the vagus nerves on the phenomenon. The results of such an observation are shown in figure 2. Before section of the nerves both the rate and the depth of breathing increased gradually after connecting the trachea with the closed system whereas after the section only the depth increased. In two minutes the minute volume of respired air increased from 585 cc. to 1420 cc. with intact nerves and from 375 cc. to 750 cc. when these were cut. This result shows that a reflex through the vagus nerves cannot be responsible and we are driven to conclude either that afferent pathways from the respiratory muscles must be concerned or that some at present inexplicable change in the hormone stimulus is the cause of the hyperpnea.

CONCLUSIONS

After section of the vagus nerve in decerebrate cats, the breathing usually declines somewhat in minute volume and the rate diminishes, often in some simple ratio to the normal rate. Similar section in decerebrate rabbits causes complete breakdown of the respiratory function. Increasing the percentage of carbon dioxide in the inspired air has almost exactly the same stimulating effect on respirations before and after section of the vagi, the only difference being that with high

Digitized by Google

percentages of CO_2, the respirations after section of the vagi become slower and the minute volume ceases to increase and may decline.

The excitability of the respiratory center to afferent nerve stimulation (sciatic and vagus) is not definitely increased after the intravenous injection of fixed acid or during the hyperpnea induced by respiring atmospheres rich in carbon dioxide or atmospheres poor in oxygen.

Conversely it is not decreased after the injection of sufficient amounts of sodium carbonate to lower the H-ion concentration of the blood.

These results show that the reflex excitability of the respiratory center is not altered by changes in the respiratory hormone (CH and CO_2-tension) of the arterial blood.

The gradual increase in breathing, which occurs immediately after connecting the trachea with a closed system of wide bore tübes, still occurs after section of the vagi.

BIBLIOGRAPHY

(1) Macleod: Trans. Roy. Soc. Can., 1919, lxxxv.
(2) Stewart: Physiology (8th ed.), 1918, 276.
(3) Scott: This Journal, 1917, xliv, 196.
(4) Scott: Journ. Physiol., 1908, xxxvii, 301.
(5) Cohen: Journ. Pharm. Exper. Therap., 1918, xi, 221.
(6) Collip: Journ. Physiol., 1920, liv, 58.
(7) Jacobs: This Journal, 1920, li, 321; liii, 457.

Digitized by Google

SENSORY STIMULATION BY SATURATED MONOHYDRIC ALCOHOLS

MARIAN IRWIN

From Radcliffe College, Cambridge, Massachusetts

Received for publication November 25, 1921

The following investigation deals with certain effects of alcohols and particularly with the question whether in a series of isomeres branching of the chain determines the relative efficiency of the members of the series.

The experiments were performed on *Allolobophora fœtida.* Each worm was stimulated by the method described in a previous paper (1). In the present investigation one worm was used for each experiment, as suggested by Crozier (2), instead of using the same worm for a series of experiments. The worm was placed on a table surrounded by a test solution, and allowed to crawl freely to the edge of the table and enter the solution. The reaction time (which is represented by the time elapsing from the moment the prostomium of the worm enters the solution until it is withdrawn) was recorded by a stop watch. The efficiency of different members of the alcohols in the series was found by determining what concentrations bring about approximately the same reaction time. Fine distinctions are not possible, because the variation in the reaction time at one concentration is too great; even when one hundred readings are made, it is difficult to determine reaction time with greater accuracy than that obtained in these experiments.

Methyl alcohol, ethyl alcohol, n. butyl alcohol, n. amyl alcohol, iso-amyl alcohol and tertiary amyl alcohol were used. As shown in figure 1, curves *M* and *E*, and figure 2, curves *I*, *B*, *T* and *A*, the efficiency of alcohols at the given concentrations is as follows: methyl < ethyl < tertiary amyl < n.butyl < iso-amyl < n.amyl.

The same order is obtained when the criterion of the efficiency of the alcohol is its ability to bring about cessation of muscular activity, as shown in table 1.

The above experiments suggest that the nature of the action of alcohols on the sensory mechanism may be similar whether it results in

Digitized by Google

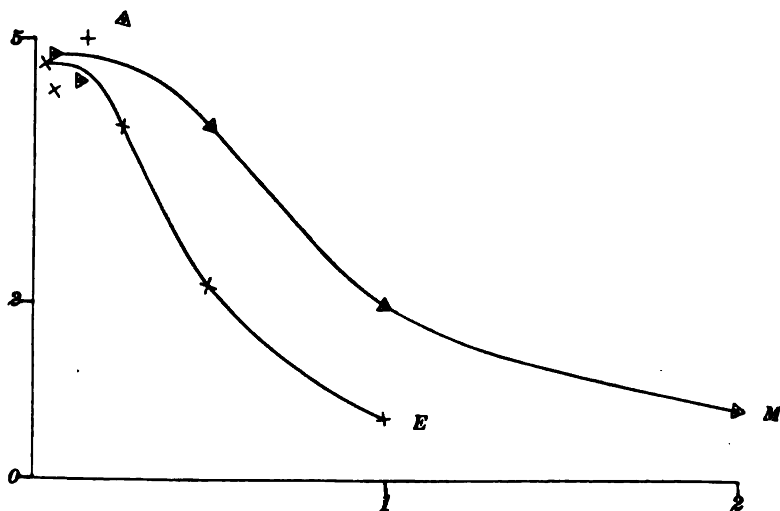


Fig. 1. Efficiency of methyl alcohol (curve *M*) and ethyl alcohol (curve *E*) as stimuli for worms. The reaction times in seconds are plotted as ordinates, and the molar concentrations as abscissae.

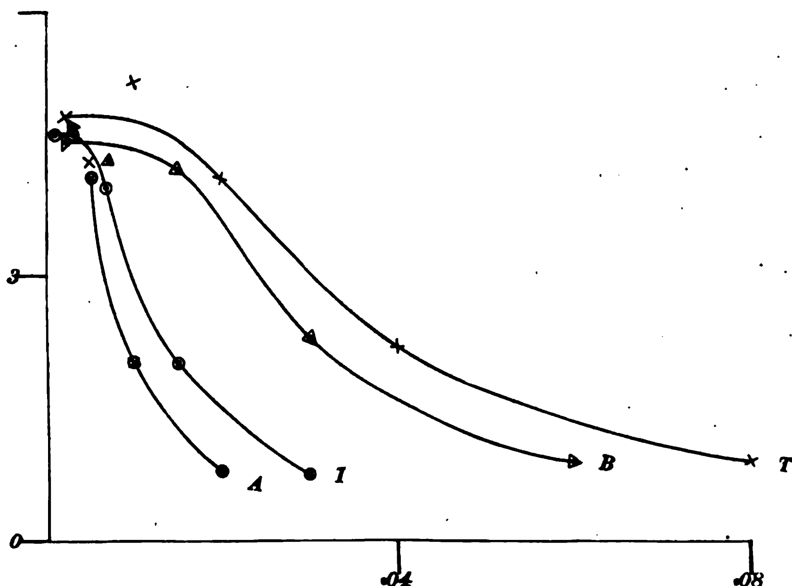


Fig. 2. Efficiency of normal butyl alcohol (curve *B*), normal amyl alcohol (curve *A*), iso-amyl alcohol (curve *I*) and tertiary amyl alcohol (curve *T*) as stimuli for worms. The reaction times in seconds are plotted as the ordinates, and the molar concentrations as abscissae.

Digitized by Google

stimulation or narcosis (3). If a certain amount of alcohol combines with a constituent of the protoplasm to produce stimulation, the combination of a larger amount may produce narcosis.

One reason why the concentration below which there is no narcosis is higher than that for stimulation is because the sensitivity of the worm decreases progressively from the anterior to the posterior portion. Therefore it requires a higher concentration of the alcohol to bring about an effect on the total body of the worm, than in the case of the prostomium, which is the most sensitive portion.

Some investigators are inclined to attribute the relative efficiency of these alcohols to their lipoid solubility (4) or to their surface tension relations (5). It may be of interest to suggest some other possibilities.

TABLE 1

Anesthetic action of alcohols on worms. Limiting concentrations below which there is no anesthesia in one hour at 23°C.

ALCOHOL	CONCENTRATION
Methyl	2.00 M
Ethyl	1.00 M
N. butyl	0.06 M
N. amyl	0.02 M
Iso-amyl	0.03 M
Tertiary amyl	0.08 M

One way of regarding the question is from a stereo-chemical standpoint (6). How far can the action of such a series of alcohols be interpreted as due to the stereo-chemical structure?

The decrease of reactivity might be interpreted as due to a steric hindrance, brought about by the arrangement of the carbon atoms in the molecule.

This idea of steric hindrance has been disputed by Michael (7), who believes that the reactivity of alcohol is independent of stereo-chemical structure, and may be more profitably interpreted from the point of view of the amount of chemical energy present.

In esterification of these alcohols, the rate of reaction decreases from amyl alcohol to methyl alcohol progressively; this is exactly the opposite of the order of efficiency obtained in the experiments described above. But when any one alcohol and its isomeres are considered, it is evident that the effect on stimulation agrees with the rate of esterification, in that the branching of the chain decreases the reactivity. Tertiary

Digitized by Google

amyl alcohol is less reactive than iso-amyl alcohol, which is again less reactive than the normal amyl alcohol. Whether the chemical effect of the alcohol is masked by a physical effect (such as solubility or surface tension relations) in the case of the normal alcohols, can not be decided at present.

It may also be suggested that the solubility of the alcohols in water may be of importance. With the branching of the chain, solubility of the alcohol in water decreases. With increase in the number of carbon atoms the solubility in water tends to decrease.[1]

Solubility in water might conceivably interfere with stimulation in a number of ways, but in view of the present state of our knowledge of this subject it does not seem profitable to enter into a detailed discussion of these possibilities.

SUMMARY

The effects of a series of monohydric alcohols on the sensory mechanism of *Allolobophora fœtida* are compared. The branching of the chain decreases the efficiency.

BIBLIOGRAPHY

(1) Irwin: This Journal, 1918, xlvii, 265.
(2) Crozier: This Journal, 1918, xlv, 315.
(3) See Höber: Physik. Chem. d. zelle und der Gewebe. 3 Aufl., Kap vi, vii.
(4) Overton: Studien über die Narkose. Jena, 1901.
(5) Traube: Pflüger's Arch., 1904, cv, 541.
(6) Stewart: Stereochemistry, 1907.
(7) Michael: Journ. Amer. Chem. Soc., 1919, xli, 393.

[1] Data regarding the solubilities of the higher alcohols in water are for the most part very unsatisfactory. N.amyl is less soluble than n. butyl, which is, in turn, less soluble than ethyl and methyl.

Digitized by Google

STUDIES ON THE RESPONSES OF THE CIRCULATION TO LOW OXYGEN TENSION

VI. The Cause of the Changes Observed in the Heart during Extreme Anoxemia[1]

CHAS. W. GREENE and N. C. GILBERT

From the Department of Physiology and Pharmacology, Laboratory of Physiology, University of Missouri, and the Department of Medicine, Northwestern University School of Medicine

Received for publication November 28, 1921

In our previous papers we have presented the changes that occur in the human during extreme anoxemia with especial reference to cardiac physiology. In our rebreather method the carbon dioxide is absorbed by shell caustic potash, hence there is no increase in carbon dioxide of the inclosed air during the augmentation of respiratory volume. In fact there may be a decrease in carbon dioxide due to the over-ventilation of the lungs with the consequent lowering of carbon dioxide tension in the body. Therefore, the gradual reduction of oxygen in the air breathed, hence in the lungs and body tissues, may be considered as the primary cause of the physiological changes observed. The condition is a true anoxemia. We have already reviewed at some length the literature of this subject especially that developed in connection with the work of the Medical Research Laboratory of the Air Service (1) where we first began the investigation of this problem on man.

In preceding papers of this series (2) we described the late cardiac effects of anoxemia when the condition is pushed beyond the ordinary limits of the technical air service examination. What we have described as the post-crisis stage includes the changes observed in the circulatory system and especially in the heart after unconsciousness has occurred. These changes in the heart of man are summarized as follows:

[1] A grant in aid of this research was made from the Patton Research Fund of the Northwestern University for which appreciation is expressed.

We also express our obligations to Prof. Frank G. Becht for the many courtesies extended by the Department of Physiology, of the Northwestern University School of Medicine, and for generous personal assistance.

Digitized by Google

1. Progressive suppression of the S-A rhythm in the descending direction to the vanishing point.

2. Establishment and persistence of the A-V rhythm with its characteristic slow and regular rate.

3. Decrease in conduction in the internodal region to the point of suppression.

4. Reversed rhythm and reversed internodal conduction.

5. Auricular pause after reversed conduction disappears.

6. These changes obey the laws of cardiac nervous control of rhythm and conduction, as outlined by Eyster and Meek.

7. Rapid recovery from all these disturbances (i.e., within a few seconds) when the man is allowed to breathe fresh air.

Most of these phenomena had been described in laboratory animals under the condition of systemic asphyxiation, but not in man. Eyster and Meek (3) especially have advocated the view that the phenomena are primarily due to vagospasm. Others, especially those working in Lewis' laboratory, have emphasized the fact that the asphyxial stress on the heart also acts directly on cardiac tissue. In all the earlier experiments on animals two factors are involved, oxygen want and carbon dioxide excess. Mathison (4) alone has tried to separate these factors by allowing his animals to breathe pure nitrogen, thus getting rid of the carbon dioxide by excessive ventilation. He concludes that "want of oxygen . . . . alone is responsible for the production of block." He says also: "the heart block is undoubtedly due to the effect of want of oxygen on the cardiac tissues." Mathison describes stimulation of the cardio-inhibitory center, especially after chloroform, but he closes his article with the sentence: "Heart-block appears to be a regular occurrence during asphyxia in dogs in which the vagi are cut. When the vagi are intact, permanent cardiac inhibition frequently comes on before heart-block can appear."

These quotations summarize the most direct evidence bearing on our problem that we have been able to find. There is nothing in the literature that deals directly with man which furnishes an experimental basis for analyzing the mechanism involved in the observations which we have reported.

Lutz and Schneider (5) studied the responses of men breathing pure nitrogen from a Larsen spirometer supplied from a gas bag and exhaling into outside air. By this method they produced acute anoxemia in a few seconds. They obtained cardiac acceleration in from 5 to 55 seconds, "within 15 seconds in sixty per cent of the cases." While they

Digitized by Google

obtained unconsciousness in certain tests they record no heart rates at this stage, in fact, resupplied air and studied the return phenomena before the extreme anoxemia with which we are concerned had appeared. For the purposes of our experiments such methods are too acute and do not allow full adjustment of the tissues to the external conditions. The same questions hold with this work as with that of Mathison on dogs.

We have not tried extreme anoxemia during atropinization, in fact we have had some hesitation in using this, the only method available for answering the question whether anoxemia in man produces the observed cardiac changes by direct action on the heart and its intrinsic mechaniisms, or by changes in the extrinsic nervous apparatus, i.e., primarily by vagospasm. We have interpreted our human results as due to the latter phenomenon. But we have not deemed it advisable to perform the crucial tests on man without further experimental data from lower animals.

In the present paper dogs are used as experimental materials. A further attempt is made to determine observational facts on which to base an answer to the questions stated above.

Method. Dogs have been used exclusively during this series of experiments. Anoxemia has been induced after chloretone anesthesia either alone or combined with ether. We have used the rebreather method, constructing an apparatus of small size suited to animals of about 7 to 10 kilos body weight. Changes in the general blood pressure have been measured by a mercury manometer, taking the pressure from the carotid artery. Respiratory rate was recorded by the movements of the spirometer, which also recorded, though with a low percentage of accuracy, the relative tidal volume. This apparatus records the progressive changes in volume of the inclosed air, thus giving a measure of the variations in rate and amount of oxygen consumed.

Electrocardiograms were taken at intervals of about 4 minutes beginning with the normal. A continuous electrocardiogram was taken from a late moment in the pre-crisis stage through the entire post-crisis stage and till the ending of the experiment by death of the animal, or by recovery following artificial respiration.

An analysis of the enclosed air in the rebreather chamber was made at the close of the experiment. The Haldane apparatus and method were used. The sample of air analyzed was taken from the inhalent tube, in the attempt to measure the composition of the air used by the animal at the last inhalation before respiratory failure. This doubtless

Digitized by Google

gives a somewhat higher figure than the average gas content of the entire rebreather apparatus. It is recognized that the alveolar air oxygen tension is slightly lower than that found by analysis of the air from the intake tube.

Chloretone anesthesia was used to render the animal immobile. Three-tenths of a gram of chloretone in oil per kilo of body weight was injected into the abdominal cavity. Animals vary slightly in their sensitiveness to chloretone. Occasionally a second small injection was required to produce sufficiently deep anesthesia. The 0.3 gram-per-kilo dose, however, produces complete unconsciousness in about 10 minutes. Chloretone in excessive doses somewhat depresses the medullary centers, but the reactions of the animal to anoxemia are qualitatively normal. We have used ether anesthesia, also chloretone-ether, but we are confident that the use of chloretone alone is without error.

EXPERIMENTAL DATA

Progressive anoxemia has been used on twenty-one animals, a total of forty-one expermental tests, to determine the course of physiological events. After anoxemia is pushed to the stage of suppression of the respiratory movements there still remains a considerable interval during which artificial respiration quickly revives the animal. Revival permits several tests on the same dog. Since our primary purpose has been to determine the mechanism of the effects of anoxemia expressed by changes in the heart, we have made the tests in four groups: *1*, with the vagi intact; *2*, with both vagi cut; *3*, after atropine; and *4*, with the vagi cut at the moment when advanced responses are in progress in the heart.

Anoxemia with the vagi intact. The dog under chloretone and with intact vagi gives a cycle of changes in response to progressive anoxemia that is characteristic and qualitatively constant. In all essential respects the physiological changes observed are very similar to those observed in man up to the stage when, in man, the tests are terminated. In the experiments recorded here the tests were carried to a much greater extreme. The changes observed in 16 experiments on intact animals which we stress are first, in the respiratory rate and tidal volume; second, the rate of oxygen consumption; third, the blood pressure; fourth, the heart rate and sequence.

The respiratory rate and tidal volume. The changes in respiratory rate and volume during general asphyxia in man and animals have been

Digitized by Google

presented in an extensive literature. The more recent papers that present aspects of anoxemial asphyxia are those from the experiments of Hough (6), Mathison (4), Gasser and Loevenhart (7), the reports of Lutz, Gregg and Schneider (8), Ellis (9), Greene, (10) from the Air Service data, and Haggard (11). The literature is more fully reviewed in these latter papers.

The experimental data concerning the respiratory responses of the dog to progressively induced low oxygen are in manuscript in a paper by the senior author (12), but we abstract by permission from the summary of that paper:

Chloretonized dogs during the rebreather test show the following panoramic changes in respiratory rate and amplitude. The sequence is more sure when the cycle is completed in from 15 to 18 minutes.

1. There is little change in either the rate or amplitude of respirations in the early part of the anoxemial test, i.e., in the first 50 to 60 per cent of the duration of the test.
2. The amplitude and tidal volume steadily increase during the last half of the test and until the respiratory crisis is reached.
3. The respiratory rate also increases but becomes more and more irregular as the crisis is approached.
4. The rate and amplitude both rapidly decrease during the post-crisis until within a few seconds all movement ceases.
5. The rate of oxygen consumption was remarkably uniform to the approach of the crisis when it progressively decreased until all respirations ceased.

The dog endures a surprisingly low oxygen, as a glance at our tables will show. The average of all the acceptable tests is 3.26 per cent. The extremes vary from 4.9 to 1.6 per cent. The highest content of oxygen of air that supported respiratory movements in the dog is well below the limits that produce respiratory stress and failure in the few extreme cases we obtained with men. Only one man of our series, Lt. S. A. (13), certainly reached the limit of respiratory pause. His residual oxygen in the rebreather was 7.1 per cent. Others, in light of our more recent experiments on dogs, were undoubtedly just short of the point of respiratory failure when removed from the test. The evidence is found in the cardiographic records.

The rate of oxygen consumption, which of course varies with the size of the animal according to the surface area, is very uniform up to the moment of the onset of the respiratory crisis. From this point until the respirations cease the rate of oxygen consumption progressively diminishes (see exper. 38 and 41, figs. 24 and 34). Following the last respiration the base line usually falls somewhat in the record (see exper.

Digitized by Google

TABLE 1

Showing the entire group of experiments of the series. Chloretone anesthesia was produced by injecting a saturated warmed solution in oil into the peritoneal cavity. To the volume of air recorded for the rebreather must be added the volume of the dead space of the apparatus, about 1100 cc., and the air of the respiratory passages. Carbon dioxide was not always perfectly absorbed in the earlier experiments.

DATE 1920	EXPERIMENT	DOG	WEIGHT	CHLORETONE PER KILO	RESPIRATIONS STOPPED	AIR AT BEGINNING	AIR AT END	OXYGEN REACHED	CO_2	VAGI CUT OR INTACT	ELECTROCARDIOGRAMS
			kgm.	*grams*		*liters*	*liters*	*per cent*	*per cent*		
5-20	1	1		None		6.0				Intact	No
5-20	2	1		None	15′ 00″	6.0	3.37*	2.44		Intact	No
5-21	3	1		None	10′ 00″	5.0		4.4		Intact	No
5-21	4	2		None		5.0		6.1		Intact	No
5-22	5	1		None	14′ 00″	6.0	3.75*	2.7	0.02	Intact	No
5-24	6	3	11.4	0.3	17′ 15″	6.0	4.5	3.83		Intact	No
5-24	7	3	11.4	0.3	14′ 30″	5.0	3.9			Intact	No
5-24	8	3	11.4	0.3	17′ 00″	5.0	3.87	3.62	0.45	Cut	No
5-25	9	5		0.3	21′ 50″	5.0	3.9	3.82	0.10	Intact	No
5-25	10	5		0.3	19′ 20″	4.0	3.12	3.71	1.65	Cut	No
5-25	11	5		0.3	19′ 45″	3.0	2.37	3.11	2.66	Cut	No
5-26	12	6	20.0	0.3	11′ 12″	6.0	4.8	4.09	4.00	Intact	Yes
5-27	13	7	13.0	0.4	14′ 00″	4.0	3.05	2.58	0.68	Intact	Yes
5-27	14	7	13.0	0.4	5′ 00″	Short special test		1.6	0.09	Cut	No
5-27	15	7	13.0	0.4	13′ 42″	4.0		4.06	1.5	Cut	No
5-27	16	7	13.0	0.3	10′ 48″	4.0	3.15	3.05	1.48	Cut	No
5-28	17	8	11.4	0.2†	13′ 00″	4.0	3.15	2.59	1.63	Intact	Yes
5-28	18	8	11.4	0.2†	12′ 15″	3.0		2.76	0.93	Intact	Yes
5-29	19	9	9.0	0.3	15′ 20″	4.0	3.25	4.46	0.43	Intact	Yes
5-29	20	9	9.0	0.3		3.0		4.44	Trace	Intact	Yes
6-4	22	11	10.0	0.3	15′ 00″	3.0	2.2	3.27	None	Intact	Yes

Digitized by Google

6-4	23	11	10.0	0.3	10′ 15″	3.0	2.25	2.34		Cut at crisis	Yes
6-4	24	11	10.0	0.3	Short special test			1.87	None	Cut	Yes
6-5	25	12	10.0	0.3	14′ 15″	4.0	3.05	5.02	1.04	Intact	Yes
6-5	26	12	10.0	0.3	13′ 30″	3.0	2.3	5.19	0.46	Intact	Yes
6-5	27	12	10.0	0.3	15′ 20″	3.0	2.37	5.52	1.37	Cut	Yes
6-5	28	12	10.0	0.3	16′ 05″	3.0	2.3			Cut	Yes
6-10	29	13	9.0	0.3	14′ 00″	3.0	2.07	2.2	None	Intact	Yes
6-10	30	14	9.0	0.3	8′ 20″	3.0	2.2	4.27		Intact	Yes
6-10	31	14	9.0	0.3	7′ 30″	4.0	3.22	5.46		Atrop.	Yes
6-11	32	15	8.0	0.3	15′ 30″	3.0	2.2	4.17	Trace	Intact	Yes
6-12	33	16	7.5	0.3	12′ 20″	3.0	2.32	4.37	Trace	Intact	Yes
6-12	34	17	10.0	0.3†	16′ 15″	4.0	3.05	2.94	Trace	Intact	Yes
6-12	35	17	10.0	0.3	13′ 42″	3.5	2.65	2.87	None	Cut	Yes
6-14	36	18	10.0	0.3†	10′ 25″	3.5	2.42	3.46	Trace	Cut	Yes
6-14	37	19	9.0	0.3	18′ 30″	3.5	2.5	2.43		Intact	No
6-14	38	19	9.0	0.3	14′ 30″	3.0	2.2	2.38		Intact	No
6-14	39	19	9.0	0.3	Short special test			2.2		Intact	No
6-15	40	20	16.0	0.3	8′ 54″	4.0	3.25	2.5		Intact	Yes
6-15	41	21	19.0	0.3	11′ 00″	4.0	2.92	1.8	0.10	Cut	Yes

* The trial face mask used probably leaked on expiration.

† Ether.

38, fig. 23). The explanation is that when the dog stops breathing the muscles relax and the chest volume decreases, forcing some of its alveolar air into the rebreather apparatus. When the respirations cease the tracheal tube is clamped off from the rebreather and the rebreather air then analyzed. The analyses therefore represent the percentage of oxygen in inspired air that just fails of maintaining respirations.

Effects of anoxemia on blood pressure and on the heart rate. Both the blood pressure and the heart rate respond typically and with fair constancy to anoxemia. The rebreather method with the taking up of carbon dioxide by the potash cartrige absorption induces anoxemia so gradually and evenly that the conditions can be repeated with great accuracy. The experimental cycle of changes constantly recur with only very minor variations.

Blood pressure changes in the intact animal. Blood pressure remains remarkably constant for the first half or two-thirds of a rebreather test on the dog. This is shown very well in experiment 38 with the vagi intact. In this test there was little or no change in the blood pressure for the first 10 minutes of a 16-minute experiment. Beginning at about 10 minutes, the blood pressure very slowly increased through 2 or more minutes, then more and more rapidly until the maximum was reached at the time of the vascular crisis. This crisis coincided very nearly with the respiratory crisis.

Sometimes the maximal blood pressure lags a few seconds after the respiratory failure. The absolute rise in blood pressure varies in different experiments. It may amount to as much as 40 or 50 per cent of the initial pressure (see exper. 41). Occasionally the rise in pressure is only slight. In all cases it occurs at the extreme stage of anoxemia. The rise in blood pressure is accompanied by an increase in pulse pressure. This change is never so great in the dog but that the diastolic phase, or minimal pressure is as great or greater than the normal. In man the diastolic pressure seldom increases to any appreciable degree during an official rebreather test but it always falls rapidly near its close if the test reaches the limit of compensation.

Events occur very rapidly in the circulatory post-crisis. The salient events are: progressive slowing of the heart and gradual lowering of blood pressure early in the collapse followed by a rapid fall in both rate and pressure later (see exper. 41).

In the crisis and during the post-crisis stages blood pressure undergoes greater and more rapid variations. The rule is that the pressure very

Digitized by Google

gradually falls during the post-crisis and until the respirations cease. The heart slows down during this phase and the pulse amplitudes increase. Consequently the diastolic pressure falls rapidly while the systolic pressure is maintained or may even rise. The maximal systolic pressure of this cycle is usually not reached until 20 to 40 seconds after respirations cease. The pressure events just described are followed by progressive and rapid decrease of both systolic and diastolic pressures with decrease in pulse rates until the pulse can no longer be distinguished and the pressure remains constant at about 15 to 20 mm. Hg. The length of time required for the entire post-crisis cycle is from 3 to 5 or more minutes after respirations cease.

Simple artificial respiration or insufflation suffices quickly to resuscitate an animal during the time when the pulse is still distinguishable on the manometric record. Later than this additional measures must be employed. Recovery of both heart rate and blood pressure when they occur are prompt, i.e., within 10 or 15 seconds.

The comparison between our results and those obtained by the methods of rapid deprivation of oxygen as practised by Mathison (4) and by Lutz and Schneider (5) is rendered difficult because in such experiments the results are brought on by immediate and rapid asphyxiation, i.e., within a few seconds. Mathison produced asphyxiation in his animals by stopping artificial respiration, and by using nitrogen gas with or without a minimal amount of oxygen, 1 to 2 per cent. In either case the transition from normal air to the asphyxial condition is sharp and abrupt. Schneider and Lutz had men rebreathe nitrogen gas into a small bag. Our animals are allowed 10 to 18 minutes to gradually exhaust the oxygen from the air they rebreathe. There is adequate time for slow and gradual adaptation.

When natural respirations stop it can be assumed that the tissues are already deprived of their free oxygen. The low oxygen content of the last inhaled air is the basis of this deduction. In anoxemia there is not such an abrupt and violent response in blood pressure as was obtained by Mathison. On the other hand, the rise in blood pressure is very gradual, generally uniform, and passes away with the same progressive type of readjustment.

Blood pressure changes when the vagi are cut. If anoxemia is induced after both vagi have been cut the blood pressure runs a course qualitatively very like that in the intact animal. The variations are chiefly those conditioned by changes in heart rate and pulse pressure. The detailed picture is as follows:

Digitized by Google

The average blood pressure does not vary during the early part of the test as much as in the intact animal. But as stress from oxygen-want becomes more acute the blood pressure rises as in the normal animal. The rise progressively increases to a maximum at the anoxemial crisis. Very little difference exists in either the rate or time of development of the crisis. The type change is shown in the last part of figure 34.

After respirations cease, sometimes a little earlier, the blood pressure slowly declines through 40 to 60 seconds. It then may show a slight increase, but finally falls rapidly through 2 or 3 minutes, then more slowly for 1 or 2 minutes more until the positive pressure of 15 to 20 mm. Hg. is reached.

If both vagi are cut the anoxemial curve of the dog never shows the enormous variations of pulse pressure during the early post-crisis stages. The response is in sharp contrast with the responses when these nerves are intact. We have never obtained confirmation of the lowered but sustained blood pressure associated with the slow heart rate, large pulse amplitude and heart block as given for the cat with the vagi cut in Mathison's experiments (see his fig. 5) (4). In dogs with vagi cut the final stages of anoxemial heart block have never appeared until the pressure approached equilibrium and the heart beats were no longer recorded by the manometer.

Changes in the heart rate in the intact animal. The heart rate is very uniform during the first 50 to 60 per cent of the anoxemial test. Unless stimuli from the outside occur, this regularity is uninterrupted. Sooner or later, varying somewhat with the animal, the heart rate slowly and gradually augments. This increase continues along with the increase in blood pressure previously described. Whether the increase in rate is the chief factor producing the rising pressure has not been determined in this investigation but Mathison speaks of vasomotor stimuli in the spinal animal. In an experiment running 15 minutes the increase in rate will be very apparent by the 10th or 11th minute. It will progressively augment to a maximum at 13 or 14 minutes. The maximum rate is associated with or slightly precedes the maximum blood pressure. This group of responses of maximal heart rate, crest of blood pressure and slowing and stopping of respirations is the complex for which we use the term crisis.

After the rise of blood pressure passes its crest and while the respirations are beginning to slow and oxygen consumption is obviously decreasing, the heart rate also begins to slow. The decrease in heart rate is very gradual at first but rapidly becomes increasingly slower

Digitized by Google

until the maximum rate is cut to a half or a third. In experiment 41 the slowing was from 161 to 44 beats per minute in 70 seconds.

If an animal with intact vagi is allowed to continue in the anoxemial state then the heart rate remains slow after the blood pressure falls, often stops for a few seconds at a time, and ultimately ceases altogether. The electrocardiographic record shows that beats continue many seconds after the manometer fails to record pressure changes. It takes an average of 3 or more minutes to run this cycle of changes after respirations cease.

Changes in the heart rate when the vagi are cut at the beginning of the test. If the vagus nerves are cut before beginning the experiment, the heart rate is of course at a higher level. However, for the first 50 or 60 per cent of the duration of the experiment there is no other change in the character of the rate.

During the last third of the experiment, passing through the crisis as indicated by the maximal blood pressure and stopping of respirations, the heart rate augments in the pre-crisis period and continues at a rapid rate during the post-crisis. There is no early cardiac slowing to the extremely low rate observed when the vagus nerves are intact. The rate remains uniform and high for from 1½ to 2 minutes after respirations stop and until the blood pressure is falling rapidly. By the moment the blood pressure has declined to one-half its earlier maximal the heart rate has become very evenly and gradually slower. It beats more and more feebly until it stops or until irregularities develop. When the heart is beating too feebly to produce any visible movement of the meniscus of the manometer the electrocardiograms show it to be still contracting in a normal sequential rhythm. It keeps this up for many seconds but ultimately block or independent auricular or ventricular beats are established and death follows.

The early cardiac slowing observed in dogs with intact vagi does not occur in our animals with vagi cut. Neither is there any evidence of change in conductivity, or block in the early phase of the post-crisis period. These come only 3 to 5 minutes later and are only revealed by the electrocardiograms.

Changes in the heart rate and blood pressure as influenced by cutting the vagi at the maximum slowing of the early post-crisis period of the intact animal. The discussion of the preceding topics clearly indicates that there are two critical times as revealed by the changes in heart rate during the post-crisis period. The first is at the time of cardiac slowing in the normal intact animal at or near the moment when respirations

Digitized by Google

stop. The second is the cardiac slowing that comes 3 to 5 minutes later in an animal in which the vagi are cut at the beginning of the experiment.

If at the moment of maximum slowing of this early period the vagi are cut then the whole situation is rapidly altered. The facts are revealed by close comparison of typical experiments, i.e., Nos. 38, 40 and 41. In these experiments we secured complete respiratory, circulatory and, in 40 and 41, electrocardiographic records without interruption through the entire post-crisis period. The vagi were cut in succession when the heart rates had dropped to between 40 and 50 per minute.

In experiment 38 the rise of blood pressure at the crisis was moderate but the heart slowing came on rapidly, the rate dropping from 176 at the crisis to 76 and then to 48 per minute. The right vagus was cut first, at 15 minutes from the beginning of the test and 45 seconds after respirations stopped. There was a sudden but momentary rise in blood pressure and an increase to a heart rate of 88, figure 23.

The left vagus was cut 40 seconds after the right. The heart rate immediately increased to 172, then 216. The original maximal rate was 174. The blood pressure at once rose to the maximal systolic pressure during the preceding period of slow heart beats, then as rapidly fell through 10 seconds, and more slowly through the next 40 seconds. Artificial respiration was then established and the animal promptly recovered.

Cutting the right vagus led to an increase of rate from 48 to 88. The high pulse pressure however continued. Cutting the second or left vagus released the heart at once to its maximum rate at the crisis. One can not escape the deduction that the extreme post-crisis slowing was a vagus phenomenon, i.e., vagal spasm, from which the heart was immediately released when the vagus nerves were cut.

Experiment 41 was also used to test the effect of cutting the vagus nerve during the early slowing in the post-crisis period. The maximal blood pressure was high in this experiment, about 50 per cent above the pre-crisis average. The heart rate slowed during the interval of 45 seconds between the maximum blood pressure and the stopping of respirations. The manometer failed to record a few beats near the moment of stopping of respirations but this does not veil the fact of the rapid and progressive cardiac slowing up to the moment when the right vagus was cut 35 seconds after respirations ceased.

For five heart beats preceding the cutting of the right vagus nerve the rate was at its lowest, 35 per minute. After one or two irregular

Digitized by Google

beats at the moment of cutting the heart remained very regular and strong at the rate of 44 per minute. This was adequate to maintain the pressure at a uniform level during the interval.

The left vagus was then cut with a minimum amount of manipulation. The heart rate immediately rose from 44 to 180 per minute. The blood pressure was momentarily increased but followed by a fall at first rapid, then more slowly, until no further heart beats could be shown in the record of the manometer. The rate was well sustained until the pressure became low. Then the rate, too, slowly declined just as in experiments when the vagi were cut at the beginning.

A continuous electrocardiographic record was maintained until no heart beats were visible by this means. The details obtained by this method are given later. No effort was made to resuscitate this animal.

Summary from the blood pressure records. The blood pressure records alone seem to prove that there are two post-crisis periods of slowing of the heart rate in anoxemia, the first a function of the vagus center, vagospasm, and the second a direct effect of oxygen-want on the heart itself. The electrocardiograms complete the evidence. We may therefore summarize the observations from blood pressure records obtained by carrying anoxemia to the complete limit of stopping respirations and heart beats.

1. The reactions of the respiratory center of the medulla become at first slow, then cease. When lack of oxygen is pushed to the death there is a phase during which the respiratory center does not receive enough oxygen to maintain its normal discharges. Finally it ceases physiological activity from true anoxemia.

2. The inhibitory centers controlling heart rate do not fail as early as the respiratory mechanisms. This is indicated by the appearance of the maximal cardiac slowing after the respiratory center has ceased.

3. The cardiac slowing in the early post-crisis stage is not due to cardiac failure, i.e., muscle and bundle failure, since it does not occur if both vagus nerves are previously cut.

4. Direct cardiac anoxemia is not adequate to suppress heart activity until from 3 to 5 minutes after respiratory failure.

5. The extreme slowing occurring after respiratory failure is promptly removed only after cutting both vagi. It is immaterial which nerve is cut first in so far as the gross rates are concerned, though differences exist in the behavior of the heart controlled by the right or the left vagus only.

Digitized by Google

6. The extreme slowing is due to vago-spasm which suppresses S-A rhythm. It is not ordinarily adequate to suppress A-V rhythm until anoxemia approaches a direct fatal effect. This slow rate therefore is an A-V rhythm released by vagus inhibition of S-A rhythm under the stress of anoxemia.

7. In extreme anoxemia when the vagi are intact inhibition may suppress the A-V rhythm. But when it occurs, a rhythmic center develops in the bundle branch, as in experiment 26, figured in plate I, figures 4 and 5.

8. Considering the heart itself, it is proven that there is an interval of from 3 to 5 minutes following respiratory failure during which cardiac beats are maintained. The rate becomes progressively slower and slower. At any moment during this interval a supply of fresh oxygen by artificial respiration is adequate promptly to recover circulatory and respiratory efficiency and remove the vagal inhibition.

9. What we have proven true for the dog checks so closely with our observations on man in the early stages of post-crisis anoxemia that we can not but believe that the mechanism of the reaction is the same in man and the dog in the final stages of progressive loss of respiratory and circulatory function.

10. It follows that in man asphyxiation by anoxemia has a considerable margin of safety provided only that a few whiffs of oxygen can be introduced into the lungs within the 3-to 5-minute intervals during which the heart continues to beat following respiratory collapse. This interval is critical and success does not always follow artificial respirations in the chloretonized dog when no other aid to resuscitation is used.

EVIDENCE FROM THE ELECTROCARDIOGRAMS

The electrocardiograms presented in the plates are all taken with the lead II. The lead was from the right shoulder to the left leg. Small nickel-plated electrodes were inserted through a slit under the skin and stitched into place for the early tests, but later nickel-plated binding posts were screwed directly into the head of the right humerus and into the shaft of the left femur. This last method proved very satisfactory and most convenient.

The normal dog electrocardiograms most often obtained are illustrated in either of the three normals in plate II, figure 8, plate IV figure 25, and plate V, figure 35. The R is very tall and the T negative or at best diphasic as in figure 35.

Digitized by Google

As anoxemia proceeds the most typical change is in the T wave. It becomes positive, then increasingly taller until at times the T is as tall as the original R (figs. 30 and 41). The maximum T is usually obtained at and following that stage of anoxemia in which respirations have just ceased. Figures 41 and 42 illustrate the change in the amplitude in the R which decreases and the S and T which both augment during extreme anoxemia following sectioning of the vagi and preceding complete cardiac anoxemial asphyxiation. This cyclic increase of the T running through the post-crisis was obtained over and over again. It apparently does not depend upon change in the position of the heart. The early experiments were performed with the animal lying on its back. But later the animal was turned to a 45° angle toward its left side. In this position the filled ventricle would tend to fall toward the left at all stages of the test.

The changes in the duration of the different phases of the electrocardiograms in the main coincide with those already described for man (1). With acceleration up to the crisis there is a perceptible shortening of both P-R and R-T intervals.

Post-crisis changes in the electrocardiograms when the vagi are intact. At the onset of the anoxemial crisis the blood pressure passes its crest and the heart rate becomes gradually slower; plate II, figures 9 and 10, plate IV, figure 27, and plate V, figure 38, all show this early slowing. This stage occurs at or preceding the moment of the stopping of respirations. Progressive slowing continues until the rate drops to one-half or one-third the normal. During the slowing the T wave greatly increases without other profound change.

Often the rate suddenly shifts, as in plate V, figure 39, to a lower level during which profound change in the type of electrocardiogram occurs. In experiment 41 the change came at the sixth complex of figure 39. The five preceding complexes show progressively longer P-R intervals, showing delayed conduction, at the sixth and two succeeding complexes S-A rhythm disappears and A-V rhythm becomes established. In the sixth complex the P wave is superimposed on the positive limb of the T. In the seventh it succeeds the T. In both cases the internodal conduction is reversed, i.e., proceeds from the A-V node toward the auricle. However, conduction is sharply delayed in the seventh complex.

The same phenomenon is shown in figure 11, plate II. The shift to A-V rhythm occurred in the second complex and those succeeding as described in the protocol of this experiment, no. 29. In plate I, figure

Digitized by Google

2, A-V rhythm was established in the third complex and continued with reversed conduction through a series of 29 beats, the last of which is shown in figure 3 of this plate.

In plate III, figure 19, a type of anoxemial influence is shown, undoubtedly of vagus origin, namely, a primary influence on the conducting bundle. A 2–1 block appeared for four groups with a progressive decrease in the P-R interval, signifying a simultaneous displacement of the rhythmic center toward the tail of the S-A node. In short, the vagus here produced its strongest effect on conduction but it also inhibited rhythm to a degree. Later in the course of the anoxemia internodal conduction was occasionally blocked and rhythm of both auricles and ventricles was enormously slowed.

The type of vagus action which drives the rhythmic center toward the tail of the S-A node is best shown in figure 28, plate IV. This figure illustrates one of a series of groups of such variations in which there was a periodic return of the normal P-R interval (see protocol, exper. 40).

The most extreme type of inhibitory displacement of rhythm is illustrated in figures 3 and 4 of plate I. After 49 consecutive beats arising in the A-V node the rhythmic focus suddenly shifts to a center in the left bundle branch, the second complex in figure 3. This type continued for 10 complexes at a rate of 26 per minute. Recovery occurred promptly on admitting air, the last complex in figure 4. The last complex in figure 13, plate III, experiment 29, also shows a rhythm proceeding from a center in the left bundle branch. In neither of these two unique cases did conduction reach the auricle.

No reference has been found in the literature to any instance of a rhythmic center so low in the bundle system. But Dr. Frank N. Wilson has very kindly sent us a very clear electrocardiogram showing displacement of the pacemaker of this class which he obtained in the dog quite incidental to other experiments.[2] "The animals were given large doses of morphine and this sometimes produced marked inhibition. In this particular animal a center located in the left bundle branch escaped and transitional complexes of the type mentioned by you occurred. In this instance I think no alternative explanation is possible." By Doctor Wilson's permission, this additional evidence is presented in figure 43, plate VI. Eyster and Meek's conception that the vagus suppresses cardiac function in the descending direction is carried a step farther by these two experiments than has previously been suspected.

[2] Private communication. Quoted by permission.

Digitized by Google

Electrocardiograms when the vagi are cut. If the vagi are both cut at the beginning of an anoxemial test, then no irregularities of the electrocardiographic complex occur in the early post-crisis period. It has already been explained that under these circumstances the rate is not slowed until late in the post-crisis asphyxiation, from 3 to 5 minutes or longer. Although the heart rate ultimately becomes gradually slower and finally stops, or becomes irregular, there is a long series of perfectly normal complexes, a series that extends through the slow and irregular rates of the early post-crisis shown in figures 1, 7, 18, 23, 24 and 34, and in the electrocardiograms of the corresponding stages. Release from these early irregularities is best shown in plates IV and V illustrating the effects of cutting the vagus nerves in succession during the early crisis.

Electrocardiographic changes when the vagi are cut during the early crisis. In plate IV, figures 29 and 30, the vagi were cut in succession at the moments indicated. When the first nerve was cut, in this case the left, there was little immediate effect on the rhythm but the P wave was changed to a negative. A-V rhythm was permanently established and conduction was apparently reversed but with occasional reversed block. Incidentally this illustrates the dominant influence of the right vagus on the rhythmic mechanism in contrast with the effect of cutting the left vagus first as shown in plate V, figures 39, 40 and 41. When the right vagus was cut first, the immediate effect was a release to S-A rhythm. However, block was established at first in the 2–1 ratio and later complete, as shown by the independent S-A and A-V rhythms persisting until the second nerve was cut, figures 39 to 41. These two experiments illustrate observations made by Cohn (13) showing the preponderance of influence of the right vagus on rhythm and the left vagus on conduction, except in our case the fact is brought out by anoxemial stimulation of the vagal center.

When the second nerve was cut in experiments of this type there was always an immediate escape to a rapid rhythm and a perfectly sequential beat that persists through several minutes, 3 to 8, before abnormality of the electrocardiographic complex was again displayed. Figures 30, plate IV, and 41, plate V, illustrate such escape.

Figures 6, plate I, 31 of plate IV, and 42 of plate V, illustrate the effects of direct cardiac final anoxemial asphyxiation. These figures present terminal stages of the series of complexes after the vagi are both cut. They are always in the late or terminal post-crisis stage of anoxemia. The first two figures show a final block of conduction with

Digitized by Google

persistence of S-A rhythm. Anoxemia here reduces the conductivity of the bundle system at a time when rhythmicity is still persistent in the upper node. The A-V node is also reduced in rhythmicity though that property is not always completely suppressed. In both experiments after a time occasional independent ventricular complexes occur. These are illustrated in figures 32 and 33.

The tracing in figure 42 illustrates the terminal anoxemia in which rhythm was first suppressed. Whether conduction was still possible could not be determined since all rhythm was suppressed.

This series of electrocardiograms on anoxemial dogs confirms our suspicion that the slowing of rate and suppression of sino-auricular rhythm in man in the early post-crisis stage is a vagus effect. This stage is entirely removed in the dog when the vagi are sectioned. Freed from vagus influence, the heart is capable of sustaining an effective rhythm for some seconds and a physiological rhythm detectable by the electrocardiograph for at least 3 to 8 minutes. The series clarifies the entire group of questions as to the relative danger in procedures which induce human anoxemial asphyxiation.

Summary of electrocardiographic changes in the dog during the post-crisis stages of anoxemia: 1. Electrocardiograms reveal the fact that the early post-crisis cardiac slowing is a strictly vagal influence on rate.

2. The degree of vagal anoxemial stimulation may completely inhibit the S-A rhythm or only drive the rhythm to a lower focus in the tail of the node.

3. When S-A rhythm is inhibited A-V rhythm becomes dominant but at a lower rate plane, 40 to 50. When A-V rhythm is established inter-nodal conduction may still persist but in the reversed direction, producing an inverted sequence.

4. Extreme anoxemial inhibition drives the rhythmic center down into the bundle branch, in the demonstrations described in this paper the left bundle branch. Rhythm may persist here with fairly regular sequence through a demonstrated series of 10 beats. Rhythm may be entirely suppressed.

5. When the first vagus nerve is cut during anoxemial vagal stimulation the type of electrocardiogram changes, according to which nerve is cut first. If the right is cut first then the S-A rhythm often reappears but interference with conduction persists so as to produce inhibitory block. If the left is cut first then A-V rhythm persists with reversed conduction or reversed block.

6. When the second vagus is cut the heart always leaps forward to

Digitized by Google

a rapid rhythm with even greater acceleration than during the precrisis stage. The electrocardiograms show that this rhythm is perfectly normal and sequential in type.

7. After a prolonged series of vagus free beats, through several minutes in experiment 40, through 400 consecutive beats in experiment 41, direct cardiac anoxemia occurs. Direct anoxemia slows the S-A rhythm as shown in all experiments, suppresses internodal conduction first as in experiments 36 and 40, or suppresses rhythm first as in experiment 41. At this stage of anoxemia the A-V center does not take on the rhythm but may occasionally discharge beats. The S-A center however is apparently last to become quiescent under direct cardiac oxygen want.

GENERAL DISCUSSION OF THE RESULTS

Early papers by Sherrington (14), Roaf and Sherrington (15), Lewis and Mathison (16), and Mathison (4) present the initial literature describing heart block as a result of asphyxia in the mammal. These authors used decerebrate, atorpinized and uninjured cats. A careful reading of their papers clearly pictures heart block as an interruption of auriculo-ventricular conduction associated with a great slowing in the heart rate. Lewis and Mathison describe prolongation of the P-R interval as introductory to simple heart block beginning with a 2-1 rhythm and leading up to complete block. They describe complete dissociation, also "a marked retardation of the auricular rate and this likewise is independent of inhibitory influences," with speedy and complete recovery. Clearly they exclude the phenomenon of inhibition. Mathison attributes heart block to "lack of oxygen rather than accumulation of carbon dioxide." He says "cardiac inhibition frequently comes on before heart block can appear," but obviously he does not associate heart block and inhibition as causal phenomena. He reports heart block in dogs when the vagi are cut.

We are unable to confirm heart block at the stage described by the above authors as a change initiated locally in the conducting tissues. Without exception our experiments on dogs have never shown the pronounced early slowing with heart block if the vagi are first cut. The initial heart block is present if the vagi are intact, absent if the vagi are cut in dogs. We agree with Mathison that the phenomenon is strictly due to a lack of oxygen. But the lack of oxygen leads to a stimulation, then suppression of respirations and to a profound increase in activity of the vagal center either overlapping or quickly following the stage

Digitized by Google

at which respirations cease. If the vagi are not injured and anoxemia is allowed to take its course without artificial respiration, there is always a composite picture ultimately showing depression of conduction to the point of block; slowing of the auricle, as we think, by inhibition of the S-A node; establishment of independent ventricular or A-V rhythm due to inhibition of the S-A rhythm; and the occurrence of bundle branch beats, all from inhibition.

If the vagi are cut then the normal high rhythm persists with sequential beats that result in sustained blood pressure for a minute or so after respirations cease. The fast rate continues straight through the early period during which anoxemial inhibition occurs when the vagi are intact.

After a more or less prolonged period, 3 to 5 minutes following the respiratory pause, and when the blood pressure approaches zero and the heart beats can no longer be readily distinguished by the mercury manometer, then a second and direct disturbance of the heart rhythm occurs. There is great slowing of the rate, heart block and independent rhythms. There is loss of auricular rhythm due to reversed block or of ventricular rhythm from direct block. Finally complete cardiac pause ensues. This seems to be the stage observed by Mathison and the onset by his methods was more abrupt than we observed.

A difficulty in correlating these facts with those related in the literature depends upon the fact that Mathison and the others used rapid methods to induce asphyxiation. The method of occlusion of the trachea suddenly withholds oxygen and fails to remove carbon dioxide, as does also the rebreathing of pure nitrogen from a bag. Our method of rebreathing purified air progressively withdraws oxygen. The rate of withdrawal used by us allows the body tissues and organs to progressively adapt to the condition of oxygen lack. There is less danger from misleading secondary reactions. On the whole a truer picture of uncomplicated anoxemia seems to result.

Mathison and others do not record normal respirations when they do occur and it is difficult to determine from blood pressures alone the corresponding times in the asphyxial cycle. Mathison's experiment 5 shows a long period of large variations in the blood pressure and a slower heart rate induced at about 60 seconds after beginning nitrogen respirations. The high blood pressure and large pulse amplitudes suggest that this phenomenon can not be the late and final direct anoxemia described by us. We are at a loss to explain the difference unless the cat and dog show a fundamental variation in this regard. We refer in comparison to our figures 1, 18, 22 and 34.

Digitized by Google

Haggard (11) has recently studied carbon monoxide asphyxiation in which the blood changes and the electrocardiographic responses were observed in animals poisoned by carbon monoxide gas. He carried experiments to fatal terminations, also recovered animals after gassing. He atropinized animals but did not operate as a method of removing vagus influence.

Haggard did not take continuous electrocardiograms but his intermittent tracings show cardiac phenomena which at one time or another we have obtained, with the exception of ventricular fibrillation. His figures 3 to 9 and 11 to 13 contain complexes that are common enough pictures in progressive anoxemia as obtained by our methods. One could interpret his results as due to true anoxemia rather than due to carbon monoxide an interpretation on which Gasser and Loevenhart based their method for inducing anoxemia. In our experiments also "the cardio-inhibitory center maintains its activity longer than does the respiratory center." Haggard (p. 398) describes a phenomenon which he attributes to "fatigued cardio-inhibitory center." We obtained some not very conclusive evidence on this point. In the preceding pages we have given the facts and explanations which will clarify Haggard's observation that after atropine and carbon monoxide "the heart maintains a rapid rate until the time of respiratory failure. Following this, the rate slowed, the P-R time increased and A-V block developed, but without the stage of auricular cessation noted in the unatropinized animals." The statement could be made of dogs under anoxemia provided we considered only the very early and the final effects of anoxemia, pictures due to two very different causes. It is apparent that Haggard missed the beautiful sequence, probably true for monoxide asphyxia as well as for simple anoxemia, by not taking continuous electrocardiograms.

We deem it more than probable that the cyle of circulatory events is fundamentally similar by the various methods of producing anoxemia. The sequence and intensity of the reactions, however, must vary with the rapidity of the onset, and with the rate and thoroughness with which oxygen is removed from the tissues. In the last and final extreme reduction of cellular oxygen suppresses the fires of physiological processes. However resistant the tissue or organ may be its activity is smothered by oxygen want.

Protocol. Exper. 26, Dog 12, Wt. 10 K. Chloretone 0.3 gram per K., air allowance 3 liters, oxygen at the crisis 4.5 per cent. Electrocardiograms.

Digitized by Google

This experiment ran through a very even and uniform pre-crisis period showing a gradual use of oxygen and little or no variations of blood pressure until the 11th minute. Blood pressure then increased until the end of the 12th minute, which marks the first maximal pressure. There was great slowing and irregularity of the heart rate but normal sequence through the maximal. At 13 minutes, 30 seconds, respirations stopped. The heart rate was progressively slowed and the pulse amplitude greatly increased. At 13 minutes, 58 seconds, the rate became suddenly very slow and continued slow through about 80 seconds. Insufflation was then begun and after 10 very slow beats recovery occurred rapidly, at 15 minutes, 45 seconds. This point is marked in figure 1 by the letter *S* over the first normal or sequential contraction in the recovery.

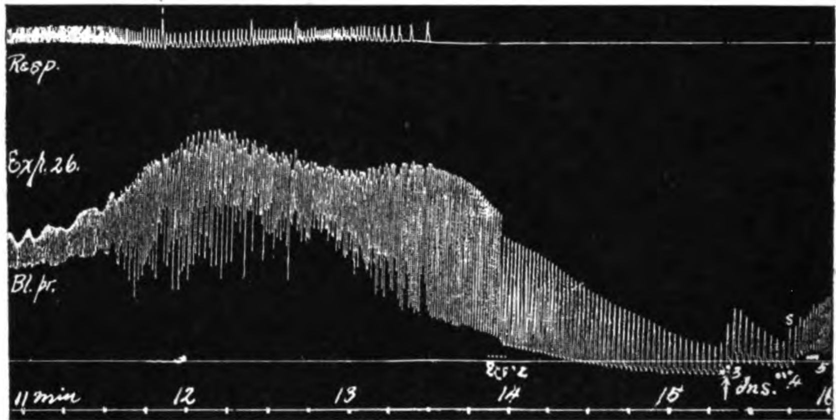


Fig. 1. Experiment 26. The blood pressure and respiratory records show a somewhat unusual form of response to anoxemia. The blood pressure crisis exhibits two periods of maximal pressure with profound slowing of respiration at the onset of the first and stopping at the second. At 13 minutes, 58 seconds, the heart beat suddenly drops to a slower rate. Plate I, figure 2, shows that this change is due to a shift from an S–A to an A–V rhythm. At 15 minutes, 20 seconds, insufflation was started. It had no influence on the electrocardiograms for 25 seconds when at 15 minutes, 45 seconds, regular sequential beats were reëstablished on the particular beat marked *S* (see also fig. 4). The 10 beats following insufflation arise from a rhythmic point in the left bundle branch. Electrocardiograms are presented of the individual beats marked by dots. Magnification × 0.56.

Continuous electrocardiograms beginning at 12 minutes were obtained (see plate I, figs. 2 to 5). The electrocardiograms showed slow and irregular rhythm but no abnormal sequences until 13 minutes, 58 seconds, when the rhythm shifted from an S–A to an A–V origin, figure 2. With the shift the Q wave appeared and was followed by an exceptionally tall R wave, 16 mm., in comparison with the normal sequential complex which in this animal showed an R of only 2 to 3 mm. At 15 minutes, 22 seconds, the origin of the rhythm shifted to a still lower point in

Digitized by Google

the A-V bundle system, figure 3. The complex from the new focus has an S wave of 10 mm. amplitude and a tall positive T wave. It is typical of left ventricular dominance but its type shows bundle origin. The focal center is apparently in the left bundle branch and remains there for the next 10 beats. After 10 beats insufflation introduced enough oxygen to bring about a normal sequential heart beat of increasing rate and final recovery.

This whole phenomenon is interpreted as vagal stimulation by anoxemia at the center. The most striking new observation is the fact that anoxemia affects the vagal center profoundly enough to drive the rhythm to a point so low as the left bundle branch.

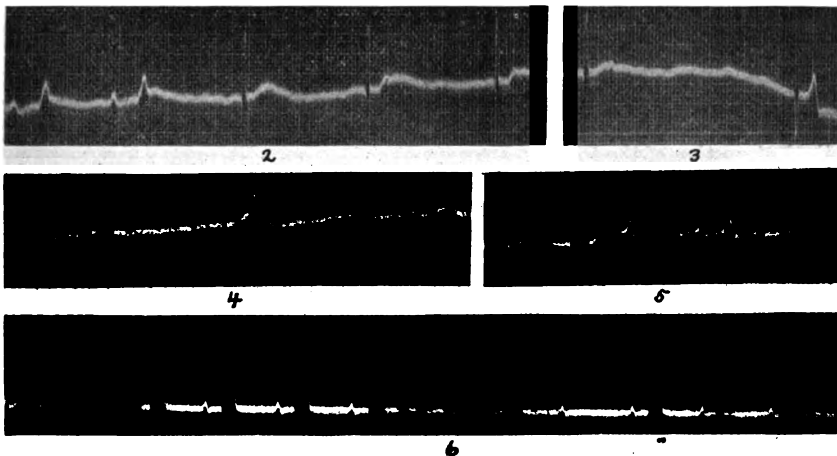


Plate I, Experiment 26. The positions of the electrocardiograms shown in figures 2 to 5 of this plate are marked in the blood pressure curve by dots under the corresponding beats. Figure 6 is the terminal record of experiment 36.

Fig. 2. Five complexes recorded at 13 minutes, 58 seconds, showing the shift in the rhythm from S-A to A-V origin. Extrinsic currents interfere but there is no very clear evidence of a P wave. Possibly the slight negative depressions in the T-waves of the last two complexes can be attributed to the auricle. There are 49 complexes in this group. Definite and clear reversed conduction characterizes the last 29.

Fig. 3. At this point A-V rhythm with reversed conduction suddenly ceased and a ventricular complex characteristic of left ventricular dominance and bundle branch origin began. This type runs for 10 successive contractions. These contractions are explained on the assumption of origin of the beat in the base of the left bundle branch.

Fig. 4. The two complexes of left bundle branch origin are followed by one sequential beat. Sequential contractions continued until complete recovery of normal rate and conduction. The sequential beats have at first a relatively long P-R interval but conduction slowly improved under insufflation.

Fig. 5. Fifth to eighth sequential beats during the recovery under insufflation.

Digitized by Google

Fig. 6. This excerpt from a continuous electrocardiographic record of experiment 36 through 7 minutes after respirations stopped shows the direct asphyxial effect on the heart when the vagi are cut. The rate progressively slowed to the auricular rate shown in this figure, 14 minutes. Then there occurred 2–1 block for two periods followed by complete block. During the last minute and a half of the entire record six independent ventricular complexes occurred. When the electrocardiographic record ceased the auricular rate was still 25 per minute and regular.

Protocol. Exper. 29, Dog 13, Wt. 9 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen at end 2.2 per cent. Vagi intact. Dog not revived. Electrocardiograms through the early and the beginning of the late anoxemial state, plate II, figures 8 to 16.

Anesthesia relatively light, occasional skeletal muscle contractions during the early stages of the experiment. Respirations rapid at the beginning but very slow and irregular from the 4th to the 8th minute and regular and typical during the last portion of the test.

Blood pressure was more sensitive to external or reflex stimulation than usual. There were two maximal pressure waves separated by 1.5 minutes, figure 7. From 13 minutes, the heart progressively slowed until at the last respiration the pulse amplitude was 70 mm. From the moment of the last respiration blood pressure fell uniformly through 2.5 minutes, when the heart beats were no longer strong enough to record. The heart rate remained uniformly slow through 70 odd seconds, then gradually increased in rate but still decreased in amplitude. At 16 minutes, the pulse cannot be counted on the blood pressure tracing, though it is clear and sequential in the electrocardiograms.

There were four respiratory gasps after regular respirations stopped. The second and third are followed by a slight increase in heart rate.

The electrocardiograms showed the usual normal—P 2 mm., Q slight, R 21 mm., S none, T negative 4.5 mm., P–R 0.098 seconds, R–T 0.200 seconds, rate 140 per minute.

At 12 minutes, 7 seconds, the T wave became positive. At 12 minutes, 23 seconds, figure 2, the T wave had increased to 6.5 mm. At 12 minutes, 53 seconds, the T had reached an amplitude of 12 to 14 mm. At about 13 minutes, 30 seconds, the slowing is more pronounced, and at 13 minutes, 42 seconds, S–A rhythm was inhibited and A–V rhythm established, figure 11, plate II. The type of reversed conduction shown in the third complex of figure 11 continues through 9 beats after which for 23 consecutive beats there was no evidence of auricular action. The 24th and 25th beats, the 3rd and 4th of figure 13, are sequential. At this point the first respiratory gasp shown in figure 7 occurred. These are followed by 10 beats with no P wave in evidence.

On the last complex of figure 13, the character of the complex changes. There is now a very short R wave, deep and profound S, and a continued tall T. This is a typical left ventricular dominance. This we also explain by assuming that at this point the origin of the rhythm shifted down the A–V node to a still lower point in the conducting system, to the left bundle branch. This type of beat gradually shifted back to the normal sequential beat as shown in figure 14. The first complex in figure 14 introduced reversed conduction. In the third complex

Digitized by Google

the P wave occurred before the ventricular complex, and in the fourth and fifth the regular sequential beats appeared and continued for some 12 beats before a second respiratory disturbance occurred. The third complex in figure 14 shows left ventricular dominance but an auricular beat occurs higher up either in the tail of the S–A node or in the atrial groove. Possibly the left ventricular type in this case can be attributed to relative right bundle block rather than left bundle rhythm. If so, it would indicate an influence of the vagus nerve on conduction extending well down into the ventricular portion of the bundle system and greater on the right bundle.

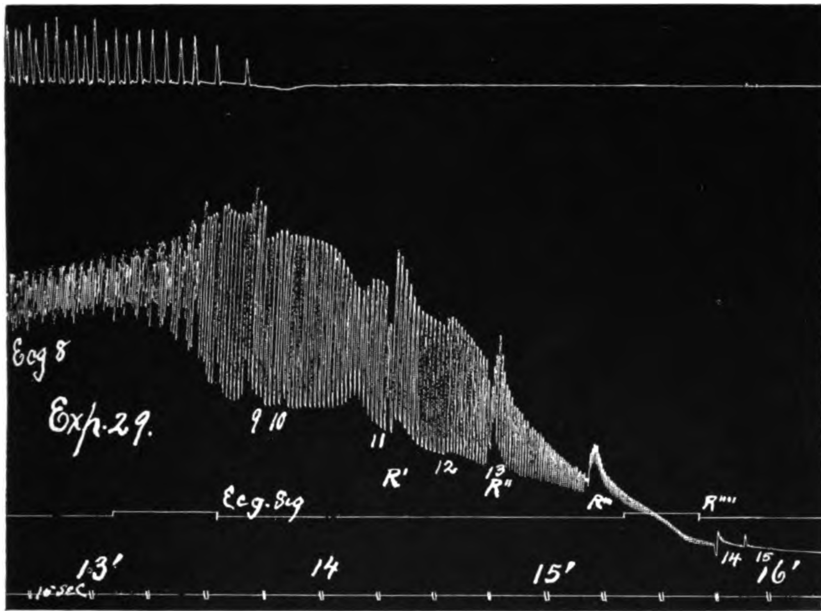


Fig. 7. Experiment 29. The respiratory and blood pressure tracings at the terminus of the test. Top line respiratory movements which ceased at 13 minutes, 44 seconds. Middle line blood pressure. The maximum or crisis occurred at 13 minutes, 30 seconds. The numbers below the blood pressure tracing indicate points figured in the electrocardiograms, plate II, figures 10 to 17 inclusive. *R'*, *R''*, *R'''*, *R''''* respiratory gasps occurred after rhythmic respirations had ceased. No attempt at recovery. Magnification × 0.68.

Sequential beats continued from 14 minutes, 30 seconds, through to 15 minutes, 50 seconds, when partial 2–1 block occurred as shown in figure 16. In the mean time the duration of the P wave and the length of the P–R interval very progressively increased to the extreme degree shown in figure 16 when 2–1 block was established. The electrocardiographic record ceased at 16 minutes with the type of record shown in figure 17, 2 minutes, 20 seconds, after respirations ceased.

Digitized by Google

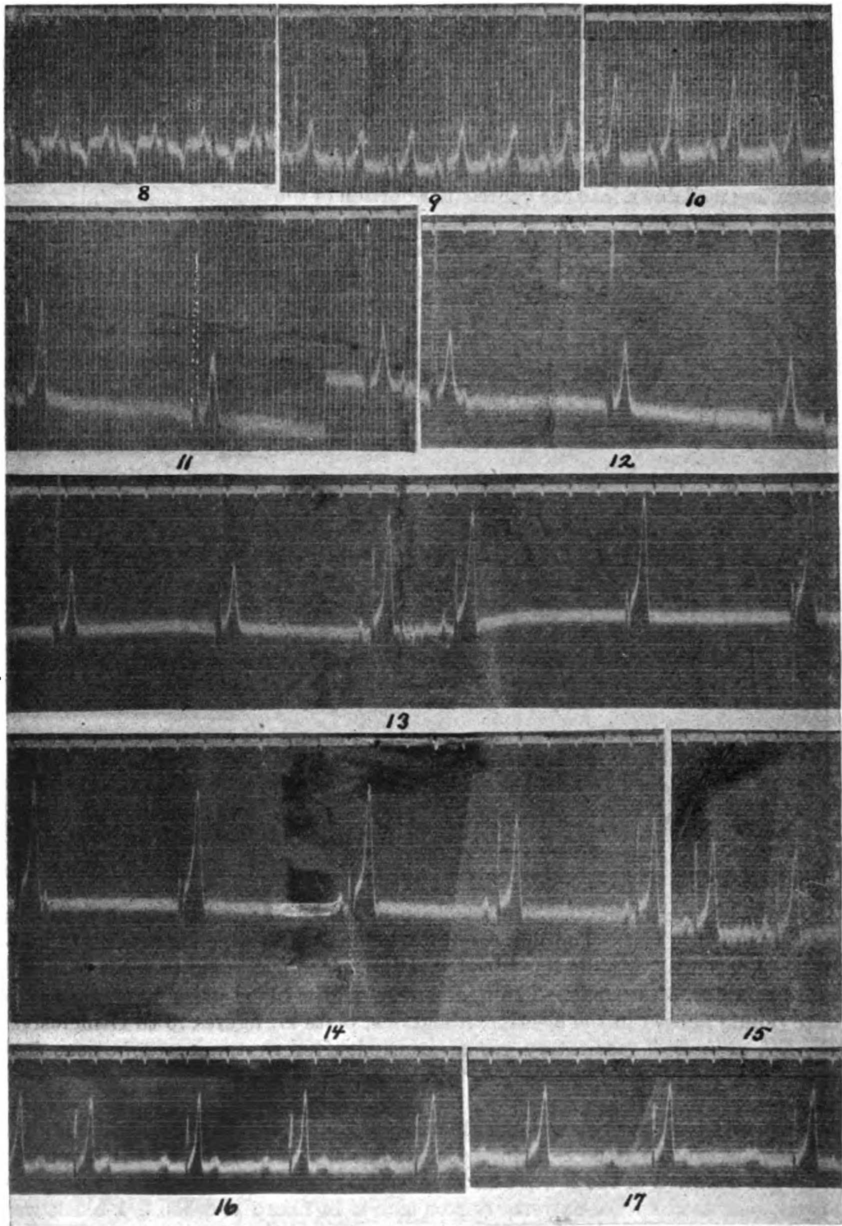


PLATE II

Digitized by Google

Plate II, Experiment 29.

Fig. 8. Normal electrocardiograms. Note the characteristic tall R and the negative T waves.

Fig. 9. Time, 12 minutes, 23 seconds. The T-wave had changed from a negative to a positive 16 seconds earlier. R now decreasing.

Fig. 10. Time, 12 minutes, 53 seconds. Both the R and the T waves progressively increased between figures 9 and 10.

Fig. 11. Time, 13 minutes, 42 seconds. Rapid inhibitory slowing of the rate (vagal) during the preceding 10 beats. S-A rhythm inhibited and A-V established at this point. Reversed conduction continued through 9 beats with block on the second, third and ninth between this and figure 12.

Fig. 12. Time, 13 minutes, 56 seconds. Reversed conduction blocked in the second complex and permanently blocked in a series of complexes following this point. Note the delay of reversed conduction in the third complex compared with the first in this figure.

Fig. 13. Time, 14 minutes, 15 seconds. Transitions occur in the pacemaker along the A-V node ending in a ventricular beat with left dominance in the last complex. The third and fourth complexes occur at the first anoxemial respiratory gasp, shown in the blood pressure curve, figure 7, and are due to momentary but partial suppression of the vagal inhibition.

Fig. 14. Time, 14 minutes, 30 seconds. Sudden transition from deep A-V rhythm to a normal sequential but slow rhythm. From this point no further irregularities in sequence occur until permanent block appeared 80 seconds later.

Fig. 15. Time, 14 minutes, 45 seconds. Momentary auricular flutter at the second respiratory gasp shown in figure 13. Sequence normal when it occurs.

Fig. 16. Time, 15 minutes, 50 seconds. Appearance of direct anoxemial block to 2-1 rhythm. For the preceding 30 seconds the P-R became progressively longer, from 0.12 second, to 0.28 second. The duration of the auricular contraction also progressively increased as shown in this figure.

Fig. 17. Time, 16 minutes. Establishment of permanent block but with both S-A and A-V rhythms still occurring. The record not taken beyond this point.

The reëstablishment of sequential beats after the extreme inhibition shown in the first anoxemial slowing indicates a partial escape from the vagal inhibition of conduction. Henderson and Haggard have given evidence indicating a similar phenomenon of escape after carbon monoxide asphyxiation.

Protocol. Exper. 33, Dog 16, Wt. 7.5 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen reached 4.37 per cent. Vagi intact. Electrocardiograms throughout the critical asphyxial stage, plate III, figures 19 to 21.

An excellent record of respirations and blood pressure was obtained with unusual features in the terminal phase. Electrocardiograms continue through the entire critical post-crisis period, figure 18, and plate III, figures 19, 20 and 21. The respiratory record shows a very uniform consumption of oxygen to 10 minutes, a progressive falling off of oxygen used until respirations ceased at 12 minutes, 20 seconds.

The rise of blood pressure was sharp during the crisis, notwithstanding the fact that the heart rate slowly decreased from 160 at 10 minutes, 40 seconds, to

Digitized by Google

128 at the time of maximum pressure, 12 minutes. There are four periods of pronounced cardiac slowing during the 13th minute, the first occurring between the last two respirations, figure 18.

At the beginning of the 4th pronounced period of slowing, marked *A–V rhythm* on the figure, auricular contractions disappeared leaving a pure ventricular complex. Occasionally there was reversed conduction with a rather long R–P interval, figures 20 and 21. Beginning with the last complex in figure 21, sequential rhythm was established, and the rate increased as shown in the blood pressure tracing, figure 18. This was possibly a release from the vagus anoxemial inhibition on account of the entrance of air obtained through insufflation begun at 13 minutes, 55 seconds. However, no recovery of the animal occurred.

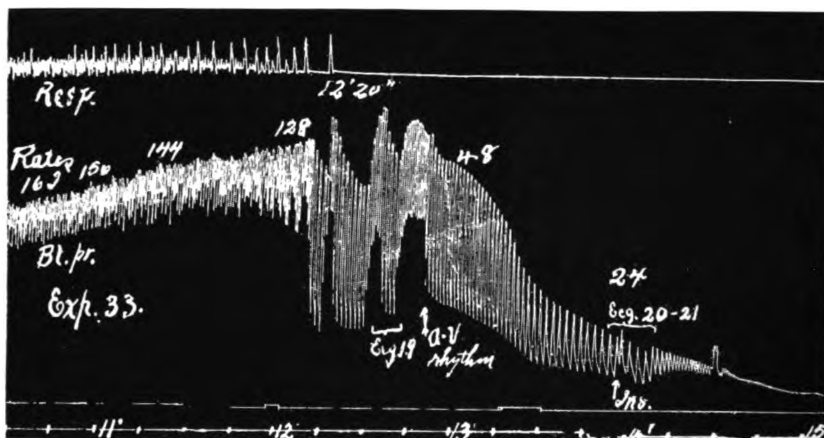


Fig. 18. Experiment 33. The terminal stage of experiment 33, vagi intact. The numbers above the blood pressure curve are heart rates per minute. At the blood pressure crisis three great irregularities in the heart rate appear, i.e., three groups of slow rates each followed by a momentary recovery. These periods are in reality 2–1 blocks as shown by the electrocardiograms, plate III, figure 19. *Ecg. 1* and *ecg. 2–3* are figured in plate III. *A–V rhythm* marks the point where the S–A rhythm was completely inhibited. *Ins.*, insufflation began. On the fifth beat, the last complex of figure 21 of the electrocardiographic series, normal S–A rhythm was reëstablished. Magnification × 0.59.

Protocol. Exper. 36, Dog 18, Wt. 10 K. Chloretone and ether, air allowance 4.5 liters, oxygen at the end 3.46 per cent. Vagi cut at the beginning. Dog not revived. Continuous electrocardiograms for 6 minutes, beginning 10 seconds before respirations ceased, plate I, figure 6.

Respirations very irregular, rather rapid until the last minute when they slowed down at the anoxemial crisis.

The blood pressure increased at the moment both vagi were cut at the beginning of the experiment and remained high until anoxemia appeared. The pressure then very gradually decreased with failing respiration. No slow beats at

Digitized by Google

the crisis, very regular heart rate with gradual decrease in pulse amplitude until the variations were no longer recorded by the manometer, figure 22. Sequential heart beats to 14 minutes, 30 seconds, plate I, figure 6. At 4 minutes, 5 seconds, after respirations stopped, complete block occurred. The auricle continued to beat with regular but decreasing rhythm for 7 minutes, 35 seconds, after respirations ceased. The auricular rate was 25 per minute at this time when the electrocardiographic record was stopped. The development of heart block in this experiment was like that in experiment 40, plate IV, figure 32.

In this experiment conduction was first eliminated in the late anoxemial asphyxiation of the tissue as in experiment 40. The electrocardiograms show that the inception of block was preceded by a group of rapidly lengthening P–R intervals. The P–R intervals in the complexes figured are 0.200 second, 0.200, 0.200, 0.208, 0.212, 0.220, 0.232, block, 0.228, block now complete. Six irregular and independent ventricular complexes occurred late after the development of block.

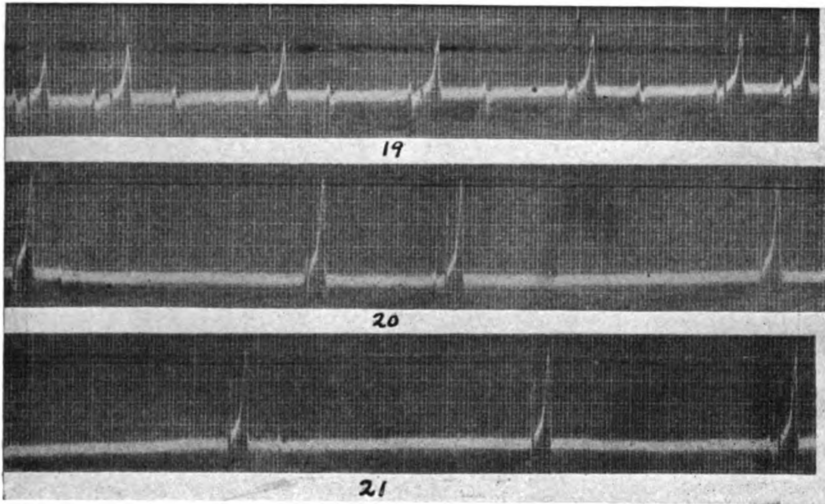


Plate III, Experiment 33.

Fig. 19. This figures the third group of four beats at a slow rate as shown in the blood pressure curve of experiment 33. Four auricular contractions are shown to be blocked and a 2–1 rhythm occurs. Recovery of conduction extended through the succeeding rapid period shown in the blood pressure curve.

Fig. 20. An A–V rhythm has persisted through the preceding 70 seconds. Reversed conduction occurs occasionally only. It is shown in the first and fourth complexes of this figure. Reversed block occurs in the second complex. The third complex is produced by an escape to S–A rhythm at the moment when insufflation began, see the blood pressure curve, figure 18.

Fig. 21. Continuation of figure 20, showing reversed conduction, block, and the permanent return of S–A control beginning with the last complex. From this point on sequence is normal and the rate progressively increases.

Digitized by Google

Protocol. Exper. 38, Dog 19, Wt. 9 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen at crisis 2.38 per cent. No electrocardiograms. Respiratory and blood pressure curves.

Respirations rapid, use of oxygen uniform, but decreasing at the very last before respirations ceased at 14 minutes, 16 seconds.

The rise of blood pressure was moderate at the crisis. Heart rate at its maximum at 13 minutes, 30 seconds, near the crest of maximal blood pressure. The heart slowing began about 30 seconds before the respirations ceased, became very profound at 14 minutes, 35 seconds, with a rate of 44 per minute. The right

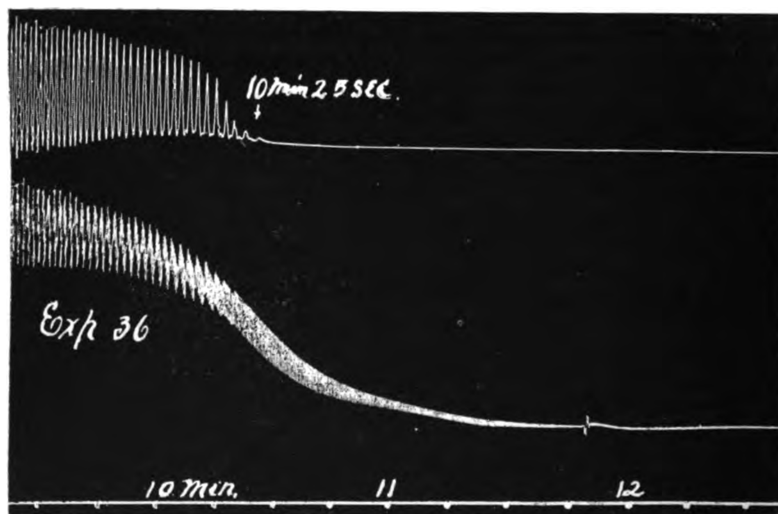


Fig. 22. Experiment 36. Showing the terminal respiratory and blood pressure records of a comparatively infrequent type of response to anoxemia when the vagi are cut at the beginning of the test. Respirations ceased at 10 minutes, 25 seconds, while the dog was inspiring 3.62 per cent oxygen and 0.45 per cent carbon dioxide. The blood pressure declined earlier than the rule but the heart rate was sustained in normal sequence for 3.5 minutes and the auricles still contracted at the end of 17.5 minutes when the record was discontinued. Figure 6, plate I, shows the beginning of direct asphyxial heart block at 14 minutes. Magnification × 0.76.

vagus was cut at 14 minutes, 58 seconds. The rate immediately doubled in partial release. The left vagus was cut at 15 minutes, 40 seconds. At this point the rate was released to 216 per minute, a greater rate than at the maximal blood pressure. During the interval between the cutting of the right and left vagus nerves the blood pressure was relatively high and the pulse amplitude great (see fig. 23).

Artificial respirations were established before anoxemia had advanced to the second asphyxial stage, natural respirations returned at 17 minutes, 15 seconds.

Digitized by Google

Protocol. Exper. 40, Dog 20, Wt. 16 K. Chloretone 0.3 gram per K., air allowance 4 liters, oxygen at end 2.5 per cent. Electrocardiograms. Vagi cut during the cardiac slowing following the respiratory crisis. Insufflation but no recovery.

The blood pressure was very uniform and even until the 6th minute when the pressure began to rise and the heart rate to increase. The maximum pressure was reached at the moment when respirations stopped, although the average high pressure was maintained one minute and more longer.

Between the last two respirations 22 heart beats occurred. Following the last respiration there are 56 beats to the point marked *left vagus cut*, figure 24. These groups are each slower than the preceding. At the last group of 12 beats the blood pressure was 158 and the pulse amplitude 80. When the left vagus was cut slow swinging pulses occurred to the point marked *R. V. cut*. There are four slihgt

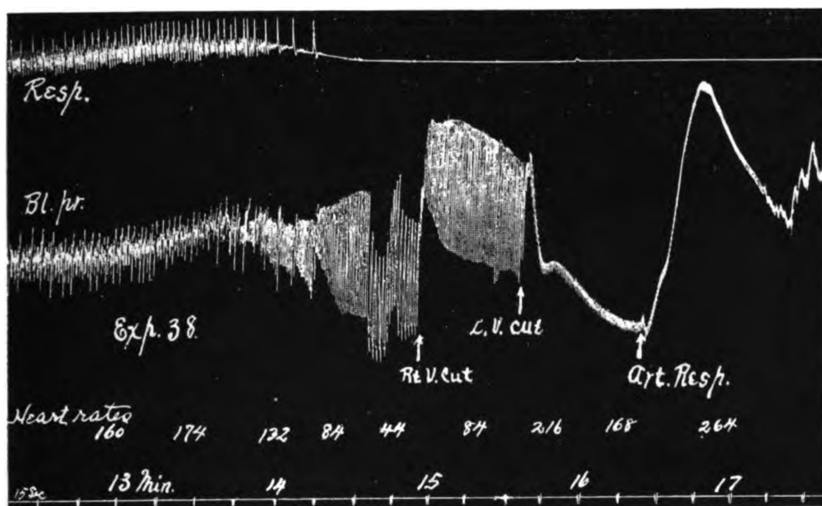


Fig. 23. Experiment 38. Slowing of the heart rate began at 13 minutes, 40 seconds, and at 14 minutes, 30 seconds, had dropped to 44. The right vagus was cut at the point marked. There was an immediate increase of the rate to 88 per minute. When the left vagus was cut the rate immediately escaped to the precrisis figure and rapidly ran up to 216 per minute. No electrocardiograms were obtained. The heart rates are given on the tracing. Magnification × 0.54.

irregularities in this series, otherwise they are remarkably even, though the pulse amplitudes progressively decreased. Counting the four irregularities there are 62 beats in the interval. When the right vagus was cut the pressure was 122 with pulse amplitudes of 68. Instantly the heart rate increased and the pressure struck a maximum of 130 rapidly falling to 108 in 8 or 10 seconds. After a small group of very irregular pressures a very regular series of heart beats and even pressure variations occurred through 25 seconds, the pressure at the beginning averaging 86. At the end of this regular group the pressure was 76. Insuf-

Digitized by Google

flation then produced irregularities in the blood pressure which however continued to fall. No recovery was obtained.

The normal electrocardiogram did not vary from the usual type. The R was tall, 23 mm., and T diphasic with the negative wave moderate. This type continued through the records of the 4th and 8th minutes. Continuous electrocardiograms began at 8 minutes, 55 seconds, and ran through the entire post crisis. At the beginning of the continuous record the T wave was positive, 9 mm. in amplitude. By counting the regular beats corresponding to the first, second and third blood pressure groups preceding the cutting of the left vagus, it was easy to identify the irregularities in the electrocardiograms. They are associated with a series of progressive shortenings of the P–R intervals. Measuring straight through the three irregular periods shown in the blood pressure record before the left vagus was cut we have the following P–R times in order: 1st beat,

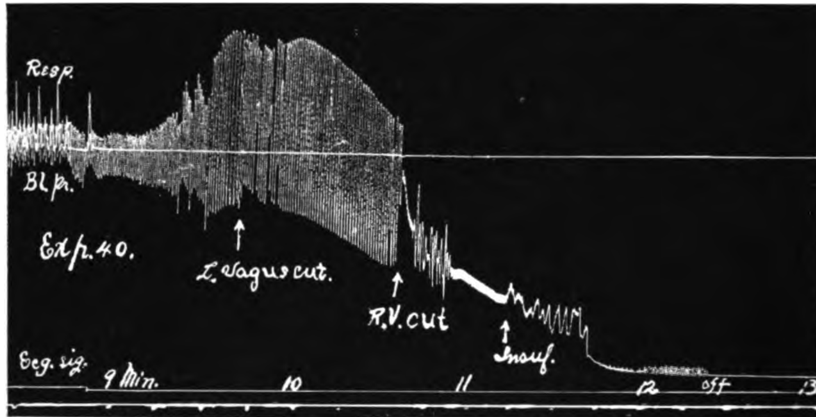


Fig. 24. Experiment 40. Respirations stop at 8 minutes, 56 seconds. Fifty seconds later the left vagus was cut. At 10 minutes, 35 seconds, the right vagus was cut. *Insuf.*, insufflation began but changed to bellows and stopped at *off*. No recovery. The electrocardiographic record was continuous from 8 minutes, 54 seconds, to 17 minutes, 40 seconds. At 12 minutes, 50 seconds, independent auricular and ventricular rhythms, i.e., complete block was established. At 14 minutes, 40 seconds, the auricular electrocardiograms were still regular but became too weak to photograph. At 17 minutes, 40 seconds, the ventricle was contracting irregularly at about 6 per minute. The record was then stopped at 7 minutes, 46 seconds, after natural respirations ceased. The time line is raised to 30 mm. pressure. Magnification × 0.59.

0.112 second; 2nd, 0.100; 3rd, 0.092; 4th, 0.080; 5th, 0.084; 6th, 0.064; 7th, 0.072; 8th, 0.052; 9th, 0.012; 10th, 000; 11th, 0.128; 12th, 0.100; 13th, 0.112; 14th, 0.108; 15th, 0.092; 16th, 0.052; 17th, 000; 18th, 0.136, 19th, 0.116; 20th, 0.100; 21st, 0.088; 22nd, 0.056; 23rd, 0.020; 24th, −0.040 (reversed conduction); 25th, 0.112; 26th, 0.092; 27th, 0.088; 28th, 0.084; 29th, 0.120. These conduction times identify the irregularities as due to a progressive displacement of the rhythmic center in the

Digitized by Google

descending direction the most striking in our series. The 10th, 17th and 24th are the critical complexes, plate IV, figure 28.

At the point marked, plate IV, figure 29, the first or left vagus was cut. The two beats preceding the section of the nerve have buried P waves, so also the first beat following the cut. The third complex shows a well-marked reversed conduction, the P wave occurring late in the T. This auricular complex began a series of negative P waves running through the entire group of electrocardiograms until the second or right vagus was cut. The 5th beat showed a reversed conduction time of 0.216 second, in the sixth beat the R–P interval is 0.328 second, if indeed this P should not be considered as belonging to the following complex. The next 5 or 6 contractions introduce variations of similar type, and this phenomenon recurred at intervals until the second vagus was cut. Certain ventricular complexes show no associated auricular contractions.

At the intervals between the 58th and 63rd beats after cutting the left vagus, there are variations in the iso-electric period which mark the lifting and cutting of the right vagus nerve. Although the nerve was cut promptly the exact point of cutting is in doubt. The 64th beat is partially recorded only. The 65th beat and the series that follow are normal sequential complexes increasing very rapidly in rate and decreasing in the amplitude of the T, through 18 or 20 beats. The 18th recovery beat is at a rate of 242, P–R 0.104 second, R–T 0.104 second, P 1.6 with rather broad base, Q 1, R 17, S none, and T 7. After the 22nd beat there was some irregularity in the sequence.

For about 40 seconds following the section of the second vagus the record was regular and uniform with slight broad wavelike variations (suggestive of some extrinsic influence). At the stage of anoxemia when these end the T waves greatly augment, changing from 6 to 10 mm. in about 10 beats. The P–R interval lengthens to 0.14 second. At the end of the tracing the T wave had increasd to 16 mm. and the P–R to 0.16 second and the rate had slowed to 106 per minute. These complexes are regular and slow sequential beats with tall T waves and increasingly long P–R intervals. The 10 complexes preceding complete and final block have P–R intervals that measure 0.136 second, 0.152, 0.152, 0.160, 0.160, 0.168, 0.220, 0.232, block, and 0.248. All succeeding contractions are blocked, figure 31. A regular auricular rhythm continued through about 2 minutes but the P waves became increasingly faint until they could not be distinguished at 14 minutes, 50 seconds.

Occasional ventricular complexes occur during this time. The first one is fused with an auricular complex, the second, third and fourth are obviously independent, and fifth appears so but follows a P wave by 0.092 second, the 6th and 7th follow P waves by 0.180 second, the 8th by 0.008 second, and in the 9th P is buried in the ventricular complex. The ventricular rhythm is obviously wholly independent.

The auricular rate dropped from 100 to about 26 per minute during the time of block. Ventricular complexes only are visible throughout the electrocardiographic records of the 5th and 6th minutes after respirations ceased. These are at a low but fairly regular rate, about 12 per minute at first but 6 per minute in the tracing which closes our record, figure 33.

The disturbances following the stopping of respiration can in this case all be attributed to vagospasm. They disappear on cutting both vagus nerves. The

Digitized by Google

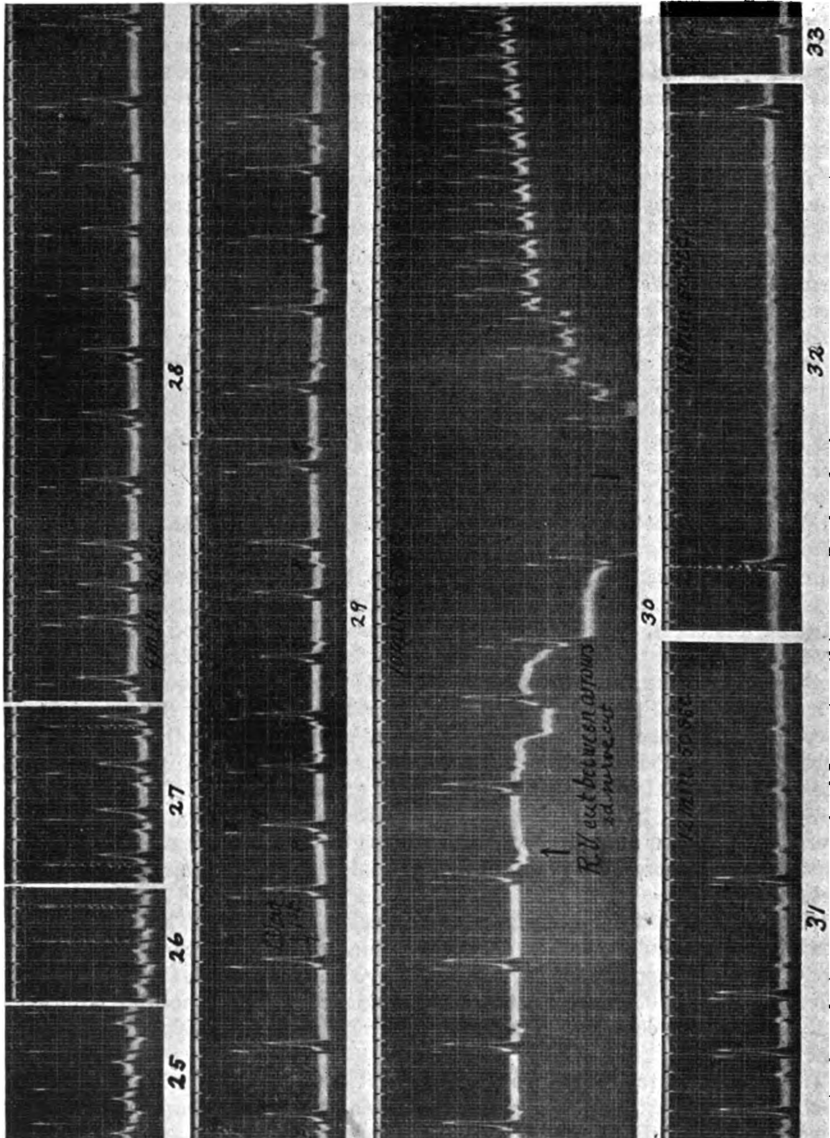


Plate IV

Digitized by Google

sequential rhythm that returns is perfectly comparable to that of experiments in which the vagus nerves were cut before anoxemial asphyxiation began. In this experiment when the heart itself became asphyxiated sequential beats were suddenly stopped by block. The auricle continued in regular rhythm through many seconds until finally the auricular complex became too faint to be distinguished. In the mean time independent ventricular complexes at long but comparatively regular intervals appeared and persisted to the end of our record. The appearance of augmented T waves with the onset of the period of slowing of the heart from cardiac tissue anoxemia was typical of the course of other experiments.

Insufflation used late in the test when blood pressure was low but while normal electrocardiograms were running was unsuccessful toward reviving the animal, in fact had no observable effects.

Protocol. Exper. 41, Dog 19, Wt. 19 K., Chloretone 0.3 gram per K., air allowance 4 liters, oxygen reduced to 1.8 per cent, electrocardiograms, blood pressure, vagi cut at the crisis.

Respirations comparatively regular for 6 minutes then rapid and irregular to 7 minutes, increasing rate and amplitude to 9 minutes, decreasing amplitude 9 to 11 minutes, decreasing rate 10 to 11 minutes. Respirations cease at 11 minutes. For the first 6 minutes deeper individual inspirations occur about every 6th respi-

Plate IV, Experiment 40.

Fig. 25. Normal electrocardiograms, showing negative T waves.

Fig. 26. Seven minutes, 30 seconds, from the beginning of the anoxemial test.

Fig. 27. Eight minutes, 55 seconds, from the beginning of anoxemia. End of respirations. T wave became positive at 8 minutes.

Fig. 28. Nine minutes, 30 seconds. Periodic inhibitory displacement of the rhythmic center in the descending direction, each period ending in apparent block but probably buried P waves.

Fig. 29. The left vagus was cut at the mark *L. V. cut*, 9 minutes, 50 seconds. In the third complex the P is inverted and conduction is retrogressive. From this point through the interval before the cutting of the second vagus the P wave was always inverted, conduction was reversed and occasionally there was reversed block.

Fig. 30. At 10 minutes, 35 seconds, the second or right vagus was cut somewhere between the points marked, probably at the second arrow. After a few beats normal sequential rhythm was rapidly reëstablished, the rate increasing through the first 10 or 15 contractions following the second arrow.

Fig. 31. At 12 minutes, 50 seconds, or 2 minutes, 15 seconds after both vagi were sectioned, complete anoxemial block appeared. The auricle continued to beat in regular rhythm from the S-A center but the ventricle ceased beating. After a long interval occasional independent ventricular complexes appeared with increasing frequency.

Fig. 32. Thirteen minutes, 50 seconds. The ventricle now contracted at the rate of 14 to 15 per minute. The auricular rate was about 90. One minute later the auricular complexes were too weak to record.

Fig. 33. The last recorded ventricular complex, 17 minutes, 40 seconds, after respirations ceased.

Digitized by Google

ration, from 6 to 8 minutes fewer deep inspirations, from 8 to 10 minutes more frequent deep gasps that become very marked near the end at 11 minutes.

The blood pressure was very uniform and even, one of the most regular records of the series. After 5 minutes it slowly and progressively increased to a maximum at 10 minutes, 25 seconds. The maximum pressure came about 40 seconds before respirations stopped but after a decrease in the use of oxygen was apparent. The blood pressure fell very slightly through 40 seconds, then somewhat more rapidly until the vagus nerves were cut (see fig. 24). Later the pressure fell promptly to the level shown in the figure. The events are figured through only 4 minutes after respirations stopped.

The heart rate began at 109 per minute. In the 5th minute it had increased to 118, 121 in the 8th, 156 in the 10th, and 161 at 10 minutes, 15 seconds. The rate rapidly fell then to 145 and finally 44 when the right vagus was cut. There were no changes in heart rate between the cutting of the right and left vagi. When the second or left vagus was cut the rate immediately increased to a maximum of 185, then decreased through the rates shown in the figure to 64 at 14 minutes, 40 seconds, after which the manometer no longer recorded, though the electrocardiograph recorded complexes for 30 seconds more, when the heart stopped completely as shown in plate V, figure 42.

When the second vagus was cut the blood pressure immediately increased, then dropped again in 5 seconds, figure 34. This was followed by a slight second rise in pressure, then a progressive decline through 2 minutes, 25 seconds, when the heart beats were no longer visible on the manometric record. The heart rates through this period were as follows: 15 seconds before the vagus was cut 11 beats, and by 15-second periods after cutting, 44, 40, 40, 40, 36, 30, 24 and 20 on the 9th period but for the 10th not visible.

The continuous electrocardiographic tracing, beginning at 8 minutes, 45 seconds, shows beside the cardiac complexes certain gross waves corresponding to the respiratory rhythm. Periodically these waves are larger and check with the recorded deep sighing inspirations shown in the respiratory record. They aid in marking the end of active respirations in the rapidly moving electrocardiographic film.

The normal electrocardiograms show the following type: the rate is relatively high, 109 per minute. The P wave is positive and sharply defined, the P–R intervals average 0.14 second. The ventricular complex begins with a sharp abrupt R wave of short duration and large amplitude. There is only a slight S wave. The T is diphasic, with a sharp negative deflection which ends in an abrupt positive, the two of about equal amplitude. The duration of the R–T interval is 0.24 second.

At 8 minutes, 45 seconds, the rate had increased to 150 with a shortening of the P–R interval to 0.112 second. At 9 minutes, 30 seconds, the rate was 156, P–R time 0.110 second. The R had increased in amplitude to 20 mm. against the normal of 17. The heart rate reached its maximum of 161 at 10 minutes, P–R 0.096 second, R–T 0.21 second.

At 10 minutes, 30 seconds, the heart rate was 159. P–R now 0.088 second, the shortest conduction interval shown in the tracing. The T wave was no longer diphasic but terminated in a sharp positive of 5 mm. From this point on until respirations stopped the T wave gradually and progressively increased in ampli-

Digitized by Google

Digitized by Google

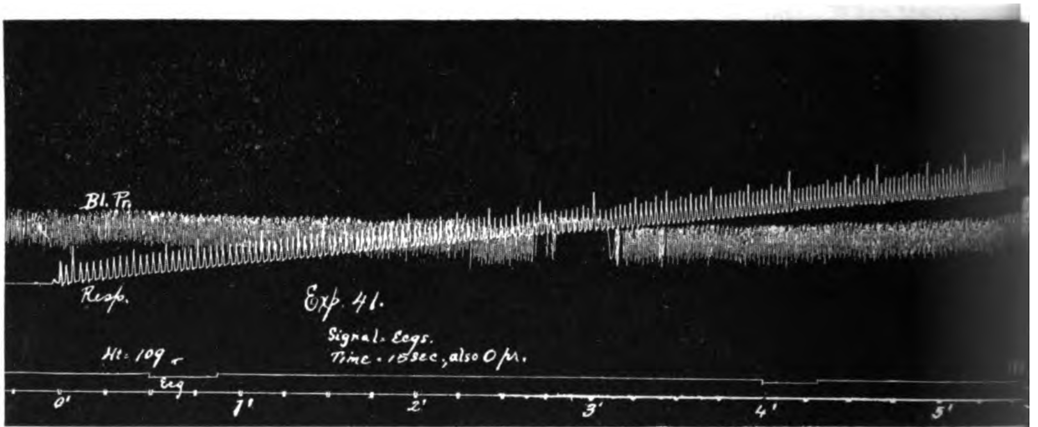
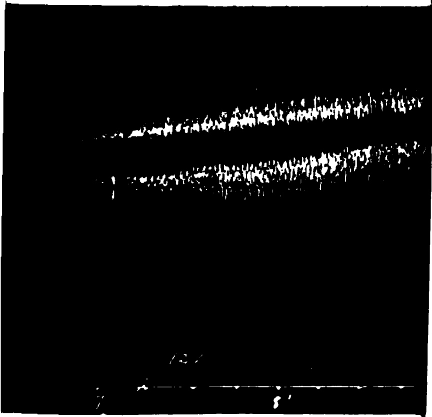


Fig. 34. Experiment 41. Blood pressure and respirations through the entire experiment. Time
in succession. No artificial respirations of any kind. Note the uniform rate of oxygen consum

Digitized by Google



…d intervals. Heart rates recorded just a…
the anoxemial crisis approached. The o…

Digitized by Google

Digitized by Google

Digitized by Google

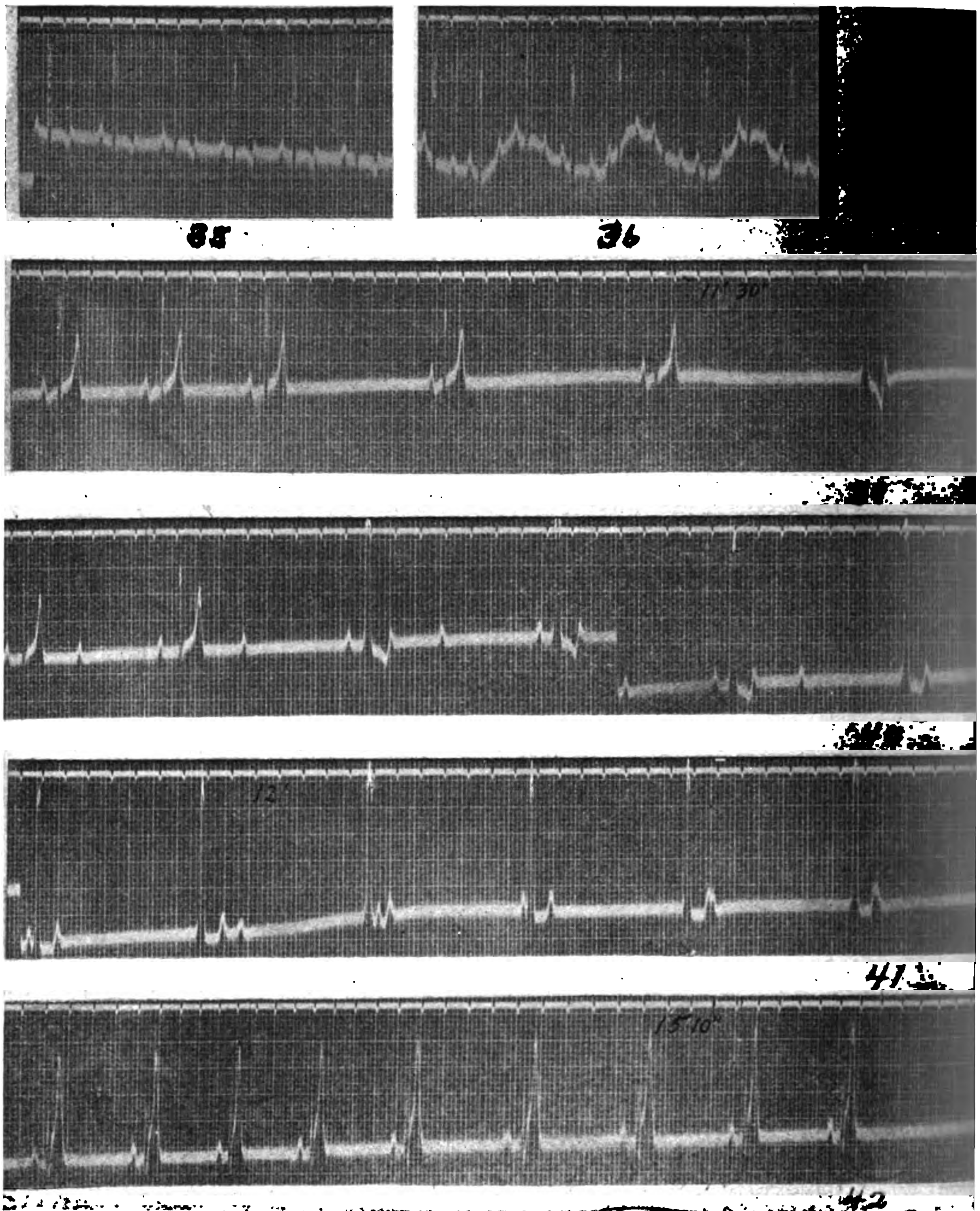


Plate V, Experiment 41.

Fig. 35. Normal electrocardiograms. The usual diphasic T wave.

Fig. 36. Time, 10 minutes, 5 seconds, from the beginning. The large waves are due to the influe

Fig. 37. Time, 10 minutes, 50 seconds. The T wave still further increased. The extrinsic curre

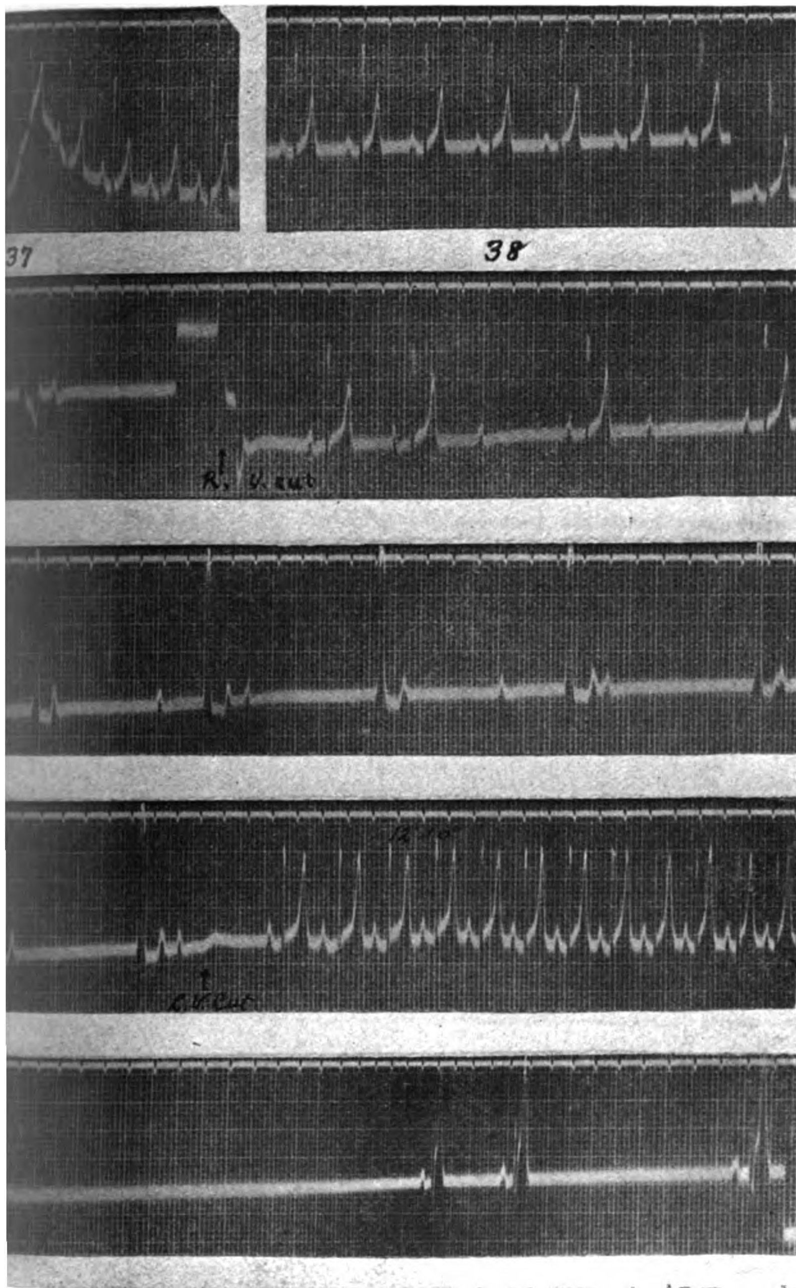
Fig. 38. Time, 11 minutes, 10 seconds. Already the inhibitory slowing of the heart is rapidly

Fig. 39. Time, 11 minutes, 30 seconds. The right vagus cut at the point marked. The first fiv node. In the sixth the P coincides with the T but falls later in the seventh, and conduction is revers 5 sequential beats immediately recur, followed by 5 groups of 2–1 block, the last showing at the be

Fig. 40. Time, 11 minutes, 50 seconds, at the point marked. The 2–1 rhythm at the beginning of interpret as arising in the A–V node as do the successive complexes in the remainder of this figure and

Fig. 41. Time, 12 minutes. The left vagus was cut at the point marked. Before the vagus was c diately on cutting the left vagus, the second, the heart began sequential beats at a rapid rate and wit

Fig. 42. Fifteen minutes, 10 seconds. This figure illustrates the last 12 complexes in the cont change in the P–R and R–T intervals at the last, the R waves progressively decrease and the S inc Compare figures 6 and 33 in which auricular complexes persist after conduction is blocked by direct



f respiratory movements. The T wave now positive.
e associated with the last respiratory gasp.
oaching. The electrocardiograms are remarkably uniform in type.
plexes are sequential. In the sixth and seventh the rhythm arises in the A–V
both complexes. When the right vagus was cut at the point marked *R–V cut*,
ng of figure 40.
figure quickly passes to a complete block. The third ventricular complex we
ure 41.
h auricular and ventricular complexes were present but independent. Imme-
intervals shortening down for the first few complexes.
s series of 400 following cutting of the left vagus. There is no appreciable
s from the types in figure 41 to these shown here, and the T waves augment.
yxiation.

Digitized by Google

Digitized by Google

tude to a maximum of 11 mm. at 11 minutes, 20 seconds, 4 or 5 seconds after the stopping of respiration. Beginning at 11 minutes, the rates computed from 10-second intervals are 145, 109 and 44. These changes in rate are accompanied by an increase in the conduction time as shown by the longer P–R intervals. The R wave decreased in amplitude through 19, 18 and 15 mm., respectively. During the 30 seconds of progressive slowing of rate the blood pressure fell. There was a corresponding increase in the pulse amplitude.

The first or right vagus nerve was cut at the point marked in figure 39, plate V, 11 minutes, 35 seconds. There were five slow swinging pulses just before the nerve was cut. Inhibition increased until the S-A rhythm gave place to an A–V rhythm, as shown in the last three complexes before the right vagus was cut. The last complex shows reversed sequence, the auricle contracting in response to A–V rhythm as in the human (2) (fig. 9, plate I). When the first or right vagus was

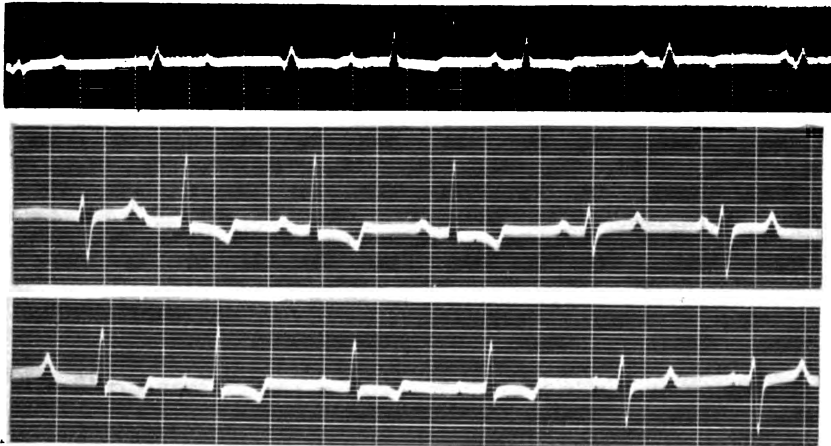


Plate VI, figure 43. Electrocardiogram showing the displacement of the pacemaker to the left bundle branch in a dog under the influence of morphine. This experiment was obtained by Dr. Frank N. Wilson and Dr. George R. Hermann, by whose kind permission it is here reproduced. The three conventional leads are shown.

cut there was temporary release from inhibition to a faster rate and normal sequential beat. After two beats a 2-1 rhythm returned for five or six groups before complete block occurred.

The ventricular rhythm during the vagospasm was very regular, rate 44. The auricular rate was absolutely irregular. The P wave was positive throughout but the P–P intervals have no regularity and cannot be lined with the ventricular complexes during this time. The intact left vagus does not inhibit the S-A nodal rhythm but it does block conduction.

On cutting the second or left vagus at 12 minutes, 10 seconds, the normal sequential type of electrocardiograms immediately returned, figure 41, plate V. The return rate was greatly augmented during the first few beats. This was

Digitized by Google

Digitized by Google

tude to a maximum of 11 mm. at 11 minutes, 20 seconds, 4 or 5 seconds after the stopping of respiration. Beginning at 11 minutes, the rates computed from 10-second intervals are 145, 109 and 44. These changes in rate are accompanied by an increase in the conduction time as shown by the longer P–R intervals. The R wave decreased in amplitude through 19, 18 and 15 mm., respectively. During the 30 seconds of progressive slowing of rate the blood pressure fell. There was a corresponding increase in the pulse amplitude.

The first or right vagus nerve was cut at the point marked in figure 39, plate V, 11 minutes, 35 seconds. There were five slow swinging pulses just before the nerve was cut. Inhibition increased until the S–A rhythm gave place to an A–V rhythm, as shown in the last three complexes before the right vagus was cut. The last complex shows reversed sequence, the auricle contracting in response to A–V rhythm as in the human (2) (fig. 9, plate I). When the first or right vagus was

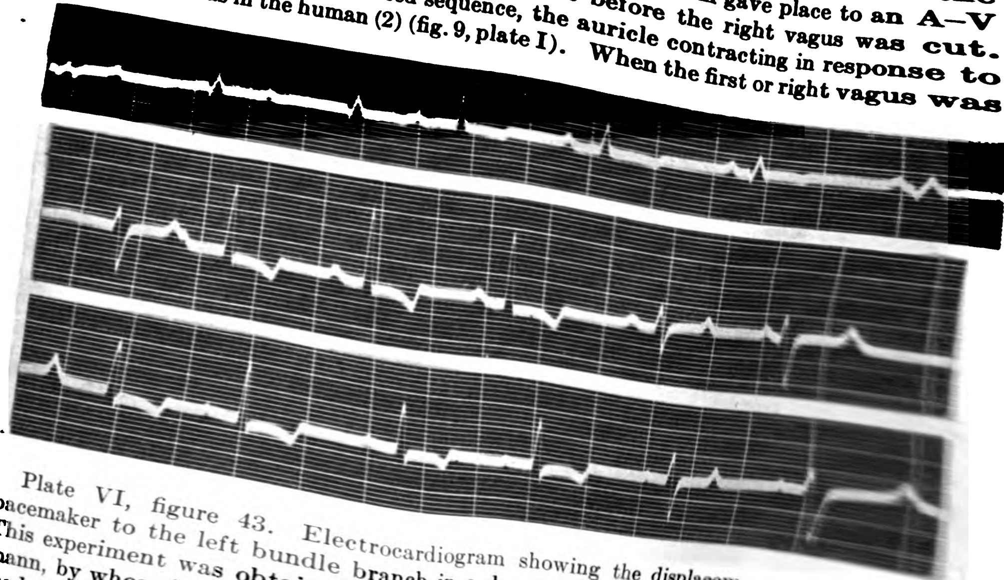


Plate VI, figure 43. Electrocardiogram showing the displacement [illegible] pacemaker to the left bundle branch in a dog under the influence [illegible] This experiment was obtained by Dr. Frank N. Wilson and Dr. [illegible] mann, by whose kind permission it is here reproduced. The three [illegible] leads are shown.

cut there was temporary release from inhibition to a faster [illegible] sequential beat. After two beats a 2–1 rhythm returned for [illegible] before complete block occurred.

The ventricular rhythm during the vagospasm was very [illegible] The auricular rate was absolutely irregular. The P wave was [illegible] but the P-P intervals have no regularity and cannot be [illegible] complexes during this time. The intact left vagus does [illegible] nodal rhythm but it does block conduction.

On cutting the second [illegible] vagus at 12 minutes [illegible] sequential type of elect[illegible] [illegible]ns immediately returned [illegible] The return rate was g[illegible] [illegible]ted during the first [illegible]

without change in the P-R and R-T intervals but with a tremendous increase in the amplitude of the T wave.

Sequential beats after sectioning the second vagus ran a continuous series for 400 consecutive beats before rhythm suddenly ceased as shown in plate V, figure 42. During this series the rate progressively decreased. The electrocardiograms were remarkably regular and uniform in character. However one striking phenomenon recurred here, namely, the augmentation of the T wave as direct cardiac anoxemia advanced. This phenomenon begins in this test early, by the reversal of the normal initial negative T. The amplitude rapidly increased at about the time respirations stopped. The T wave took on the tall type characteristic of A-V nodal rhythm during the vagospasm. When the second nerve was cut the T waves were at once almost as tall as the R waves and became taller to the end while the R waves progressively decreased.

BIBLIOGRAPHY

(1) Greene and Gilbert: Arch. Int. Med., 1921, xxvii, 517.
(2) Greene and Gilbert: This Journal, 1921, lvi, 475. See also Arch. Int. Med., 1921, xxvii, 688.
(3) Eyster and Meek: Heart, 1914, v, 119, 227.
(4) Mathison: Heart, 1910, ii, 54; Journ. Physiol., 1910, xli, 416.
(5) Lutz and Schneider: This Journal, 1919, l, 327.
(6) Hough: This Journal, 1911, xxviii, 369.
(7) Gasser and Loevenhart: Journ. Pharm. Exper. Therap., 1913, v, 239.
(8) Lutz, Gregg and Schneider: This Journal, 1919, l, 228, 280. See also Gregg, Lutz and Schneider: 216, 302.
(9) Ellis: This Journal, 1919, l 267.
(10) Greene: In manuscript
(11) Haggard: This Journal, 1921, lvi, 390.
(12) Greene: In manuscript.
(13) Cohn: Journ. Exper. Med., 1912, xvi, 732.
(14) Sherrington: Journ. Physiol., 1909, xxxviii, 381.
(15) Roaf and Sherrington: Quart. Journ. Exper. Physiol., 1910, iii, 209.
(16) Lewis and Mathison: Heart, 1910, ii, 47.

Digitized by Google

INFLUENCE OF BLOOD SERUM ON THE COAGULATIVE ACTIVITY OF TISSUE EXTRACTS

C. A. MILLS AND STEWART MATHEWS

From the Biochemical Laboratory, University of Cincinnati and the Cincinnati General Hospital

Received for publication November 28, 1921

While attempting to develop an anti-serum for tissue fibrinogen (thromboplastin) and for our tissue anticoagulant (protein fraction of tissue fibrinogen) we happened on the unexpected discovery that normal rabbit serum possesses in a very marked degree the property of rendering more intense the coagulative action of tissue extracts. This increase (being as high as about thirty-fold in one case) was only temporary, later giving way to a decrease in coagulative activity, but was so striking while it lasted that it led us to wonder whether it might not be of considerable interest in regard to the intravenous injections of supposedly non-toxic substances.

There has been considerable dispute in the past as to the action of serum on organ extracts, some claiming a detoxicating action and others an increase in toxicity. Leo Loeb (1) in 1905 reported experiments showing serum to possess the power of reducing the coagulative activity of tissue extracts. He found that previously heating the serum to 56°C. or 80°C. destroyed this action. Freund (2) in 1909 showed that the toxicity of placental press fluid was destroyed by digesting with human serum at 37°C. for one hour. That is, a toxic dose for a rabbit intravenously became non-toxic after such digestion. Dold (3) made a similar observation in regard to saline extracts of various organs. He found that, while digestion for one hour at 37°C. with fresh homologous serum detoxicated organ extracts, digestion for a similar period with serum heated to 60°C. for one hour was without influence on the toxicity of the extracts. A number of investigators (4), (5), (6), (7), (8), (9), (10) reported findings similar to those of Dold. Gley noted that digestion with serum for only 30 minutes reduced the

Note: This work was supported in part by financial aid from the Hygienic Laboratory of the U. S. Public Health Service, and the Metropolitan Life Insurance Company of New York.

Digitized by Google

toxicity only slightly, while digestion for one hour reduced it markedly. Roger found that the entire toxicity was present immediately after mixing and that detoxication occurred only after keeping the mixture at 38°C. for one hour. Giafami reduced the toxicity of placental extracts for rabbits by digestion with serum, especially serum of pregnant women. Fetal serum and horse serum he found to be much less efficacious. Schenck also worked with placental press fluid and obtained detoxication even by 15 to 30 minutes' digestion with normal serum.

There have been found results contrary to those mentioned above, however; Ascoli and Izar (11) contended that non-toxic doses of organ extracts could be rendered toxic by digestion with serum for 30 minutes at 37°C. and then keeping for 12 hours at room temperature. Heating the serum to 56°C. for 30 minutes previously they found to prevent this development of increased toxicity, as did also Berkefeld filtration of the tissue extract. Dold and Ogata (12) in repeating their previous work were unable to agree with Ascoli and Izar, but adhered to Dold's previous findings. Cesa-Bianchi (13) was also unable to decrease the toxicity of organ extracts by serum digestion or other biological procedures. He also found, instead, a slight increase in toxicity when digesting with heterologous serum.

From a study of our results set forth in the following pages it will be apparent why different findings may be had, the effect of serum depending greatly on the time elapsing before testing the mixture. Loeb also stated that the effect of the serum was a function of the time and temperature, but he found only a detoxicating action with no tendency toward increased toxicity at the start.

Experimental. The determination of the coagulative activity of the extracts was made in all cases by use of citrated horse plasma. Horse blood was drawn into vessels containing sufficient potassium citrate to make a final concentration of 0.5 per cent, the corpuscles allowed to sediment in an ice box and the clear plasma drawn off. Tissue extracts used were made by grinding the *fresh tissue* (*lung*) with 0.9 per cent NaCl solution for 5 to 10 minutes, filtering through several layers of cheese cloth and sedimenting over night at 3°C. No definite proportions of tissue and saline solution were adhered to, so that the initial activity of extracts varied considerably. Usually the extracts were approximately saturated with the soluble tissue materials, that is, they contained about 2 per cent of coagulable proteins, four-fifths of which was tissue fibrinogen.

Digitized by Google

The samples of rabbit serum tested were obtained by drawing the blood directly from the rabbit's heart without the use of anesthetic, and, after clotting, permitting separation of the serum by standing over night in an ice box at about 3°C. The tests recorded in table 1 were carried out within 3 days after bleeding the animals.

In making the coagulation tests the normal clotting time for the citrated horse plasma was taken as the time when 1 cc. of it would clot at 41°C. after adding the optimum amount of $CaCl_2$, which was 0.3 cc. of a 0.1 per cent solution. In testing the thromboplastic action of lung extract or serum, 0.1 cc. was added to the citrated plasma and the mixture brought to 41°C. before adding the $CaCl_2$. Clotting time was always measured from the time of adding the $CaCl_2$ and agitating. In testing the lung extract and serum mixtures, equal amounts of the two were mixed, shaken well by hand and placed in the water bath in which the coagulation tests were being conducted. At intervals, as noted in table 1, 0.2 cc. of the mixture was added to the citrated plasma, followed by the proper amount of $CaCl_2$ and the clotting time taken. The amount of the mixture used for the test (0.2 cc.) was so taken in order to have present the same amounts of lung extract and serum as were used in the controls. The principle used in testing the anticoagulant-serum mixtures was identical with that just described for the lung extract-serum mixtures.

Table 1 shows the typical action of rabbit serum on rabbit lung extract.

Rabbit 1 was a normal rabbit from whose heart were drawn 20 cc. of blood for obtaining normal serum.

Rabbit 2 had received two intravenous injections of tissue anticoagulant of 6 cc. each at 6-day intervals, and then ten 1 cc. injections at 1 day intervals, the blood sample for serum being taken 5 days following the last injection. We were trying for a serum here that would destroy either the anticoagulant or active coagulant. It is seen to act just the same as serum from the normal control.

Rabbit 3 received twelve 1 cc. injections of this same anticoagulant solution at intervals of 24 hours, a blood sample being drawn 2 days after the last injection. This sample of serum is labelled (3_1). Ten further daily injections of 1 cc. were given, followed in 5 days by the drawing of a second blood sample (3_2).

Rabbit 4 received the same series of injections as did rabbit 3, but a blood sample was drawn only at the end of the series, comparable to sample (3_2).

Rabbit 5 received two 6 cc. injections of anticoagulant at 6-day intervals, and was bled 10 days after the second injection.

A study of the results recorded in table 1 will show a variety of interesting facts. *First*, in each case except one (the last one recorded)

Digitized by Google

the rabbit serum alone showed a thromboplastic action on the clotting of citrated horse plasma by calcium. Thus clotting times of 1 minute 4 seconds, 1 minute 55 seconds, 1 minute 25 seconds and 1 minute 15 seconds were obtained as contrasted with the normal time of 2 minutes 55 seconds.

TABLE 1

Citrated horse plasma	1 per cent $CaCl_2$	Lung extract (rabbit)	Anticoagulant (horse)	Serum of rabbit	Coagulation time of controls	Coagulation time after incubating serum and lung extract or anticoagulant at 41°C. for:					
						1 minute	5 minutes	10 minutes	30 minutes	1 hour	2 hours
cc.	cc.	cc.	cc.	cc.							
1	0.3				2'55"						
1	0.3	0.1			17"						
1	0.3			0.1 (1)	1'12"						
1	0.3	0.1		0.1 (1)		4"	9"	9"	16"	23"	30"
1	0.3		0.1		10'46"						
1	0.3		0.1	0.1 (1)		1'55"	1'55"	1'53"	1'59"	1'55"	
1	0.3			0.1 (2)	1'04"						
1	0.3	0.1		0.1 (2)		5"	7"	7"	10"	11"	
1	0.3		0.1	0.1 (2)		2'00"	1'40"	1'35"	1'28"	1'25"	
1	0.3			0.1 (3_1)	1'55"						
1	0.3	0.1		0.1 (3_1)		8"	15"	30"	50"	58"	
1	0.3		0.1	0.1 (3_1)		2'00"	1'55"	1'55"	1'50"	1'55"	
1	0.3			0.1 (3_2)	1'25"						
1	0.3	0.1		0.1 (3_2)		7"	12"	12"	14"	16"	
1	0.3		0.1	0.1 (3_2)		2'05"	2'05"	2'05"	1'45"		
1	0.3			0.1 (4)	1'15"						
1	0.3	0.1		0.1 (4)		4"	4"	9"	14"	26"	
1	0.3		0.1	0.1 (4)		2'26"	2'35"	2'10"	2'10"	2'05"	
1	0.3			0.1 (5)	4'05"						
1	0.3	0.1		0.1 (5)		10"	11"	12"	17"		
1	0.3		0.1	0.1 (5)		9'00"					

The serum of rabbit 5, on the other hand, was slightly antithrombic the clotting time being 4 minutes 5 seconds, or 1 minute 10 seconds beyond the normal control. *Second*, each of these sera was capable of very markedly increasing the activity of the lung extract, serum 5, which alone was slightly antithrombic, exhibiting this activating power to

Digitized by Google

a less degree than the rest. The very marked increase in thromboplastic power of the lung extract becomes even more striking when we take into consideration the slight effect that dilution has on this power. Thus in a previous paper (14) Mills has shown that in diluting lung extract with 0.9 per cent NaCl solution, as the concentration of the active material is reduced from 1 to $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, etc., the coagulative efficiency decreases as represented by coagulation times of 10 seconds, 12 seconds, 14 seconds, 17 seconds, 20 seconds, 25 seconds, 30 seconds, etc. Therefore, we see that an increase of activity by the serum causing the lung extract to bring on coagulation in 10 seconds instead of 17 seconds means an eight-fold increase in activity while a clotting time of 8 seconds would mean at least a sixteen-fold increase, and down to 4 seconds would mean more than a thirty-fold increase in the coagulative activity of the lung extract. This tremendous activation occurs very quickly, for it is in evidence even after only about 20 seconds of shaking the serum and lung extract together, even without incubation. It is possibly just this property of rabbit blood that accounts for the very great ease with which intravascular clotting may be induced in rabbits.

The third point of interest in table 1 lies in the results showing the influence of these sera upon our anticoagulant. The anticoagulant used here was a 0.9 per cent NaCl extract of horse lung which had been previously dried at room temperature and thoroughly extracted with benzene, also at room temperature. Such an extract is here seen to have lengthened the clotting time of the horse plasma from 2 minutes 55 seconds up to 10 minutes 46 seconds when used in the ratio of 0.1 cc. for 1 cc. of plasma. The influence of rabbit serum on this anticoagulant solution is seen to be an immediate neutralization, the entire antithrombic action being lost while a part of the thromboplastic action of the serum is also destroyed. The notable exception to this was serum 5, which itself was slightly antithrombic. Here there was very slight reduction in the anticoagulative action of the extract. In no case was there evident an increase in antithrombic activity from the action of the serum, similar to the activation of the lung extract previously noted. There was noticed with serum 2 and serum 4 a slight tendency toward increased thromboplastic activity as the serum and anticoagulant were incubated together.

We see, then, that rabbit serum may very markedly increase the coagulative activity of lung extract (the increase being as high as perhaps thirty-fold) although the serum itself possesses only about one-hundredth

Digitized by Google

TABLE 2

CITRATED HORSE PLASMA	1 PER CENT $CaCl_2$	LUNG EXTRACT (CALF)	HUMAN SERUM	COAGULATION TIME OF CONTROLS	COAGULATION TIME AFTER INCUBATING SERUM AND LUNG EXTRACT TOGETHER AT 34°C. FOR:							
					1 minute	5 minutes	10 minutes	30 minutes	1 hour	4 hours	6 hours	In ice box 30 hours
cc.	cc.	cc.	cc.									
1	0.3			2′40″								
1	0.3	0.05		14″								
1	0.3		0.05 (1)	2′20″								
1	0.3	0.05	0.05 (1)		10″	10″	12″	12″	13″	15″	14″	20″
1	0.3		0.05 (2)	2′20″								
1	0.3	0.05	0.05 (2)		10″	10″	13″	13″	14″	16″	15″	20″

TABLE 3

CITRATED PLASMA (HORSE)	1 PER CENT $CaCl_2$	LUNG EXTRACT (HORSE)	SYPHILITIC SERUM	COAGULATION TIME OF CONTROLS	COAGULATION TIME AFTER INCUBATING SERUM AND LUNG EXTRACT AT 34°C. FOR:					
					1 minute	5 minutes	10 minutes	30 minutes	1 hour	5 hours
cc.	cc.	cc.								
1	0.35			2′00″						
1	0.35	0.05		12″						
1	0.35		0.05 cc. Serum I	1′20″						
1	0.35	0.05	0.05 cc. Serum I		9″	10″	10″	11″	13″	14″
1	0.35		0.05 cc. Serum II	1′25″						
1	0.35	0.05	0.05 cc. Serum II		8″	9″	10″	11″	12″	14″
1	0.35		0.05 cc. Serum III	1′40″						
1	0.35	0.05	0.05 cc. Serum III		8″	9″	10″	12″	14″	15″
1	0.35		0.05 cc. Serum IV	1′35″						
1	0.35	0.05	0.05 cc. Serum IV		8″	10″	11″	13″	15″	16″
1	0.35			2′00″						

Digitized by Google

the thromboplastic activity of the original lung extract. It is very evident that the increased activity is not due merely to an addition of the effects of the two separately, for serum 5, possessing no thromboplastic action whatever, still causes an eight-fold increase in the activity of the lung extract. As yet we have no evidence as to the nature of this activation.

As a natural sequence to our findings in regard to this remarkable property of rabbit serum, we next examined human serum to see if it possessed this same property and to the same degree. Blood samples were obtained from two apparently normal individuals, and, after standing over night in the ice box, the serum was drawn off and tested. The results are shown in table 2.

Here again is noted the same serum effect, only to a milder degree than that obtained with rabbit serum. The increase in coagulative activity here is approximately four-fold. The later inhibitory phase of the serum action is slight in these cases and is later in developing than with the rabbit serum.

Hirschfeld and Klinger (15) in 1914 reported a coagulation reaction which they thought to be specific of syphilitic serum. Fränkel and Thiele (16) obtained similar results. The basis of their test depended on the inactivation of tissue extracts by syphilitic serum, the extracts losing their ability to quicken the clotting time of mixtures of normal serum and citrated plasma. In order to see if syphilitic serum would differ from normal serum by our method of testing, four samples of human serum showing 4 + Wassermann reactions were tested for their influence on lung extract, with results as set forth in table 3.

We see here that syphilitic serum reacts in all ways similar to normal human serum, and does not possess any marked power to destroy the coagulative activity of tissue extracts.

DISCUSSION

We have no theory at present to offer in explaining the remarkable property of rabbit serum (and to a less degree, human serum also) described in the preceding pages. We are offering our findings at the present time for the sake of any practical value they may have in their bearing on the question of intravenous injections of protein solutions in general, and especially organ extracts and serum.

There is a possibility that many cases of sudden reaction following serum injections have been caused by the presence of small amounts of tissue fibrinogen (thromboplastin) in the serum, the blood of the pa-

Digitized by Google

tient serving to activate the coagulant to such a degree as to make it effective. It takes relatively a much smaller amount of tissue extract to produce almost instantaneous intravascular clotting in rabbits than it does to produce maximal acceleration of the clotting of the same quantity of blood *in vitro*. Thus 0.03 cc. of a saturated lung extract injected intravenously into a rabbit causes clotting in 15 to 20 seconds, but to clot 50 to 75 cc. of citrated plasma *in vitro* at 37°C. with this speed at least 1 cc. of the same extract would be required.

In observing the influence of time on the action of the serum, we see a very possible reason for the conflicting reports of past investigators. Many had noticed that the primary toxicity of tissue extracts remained for 30 minutes after mixing with the serum, but was decreased after 1 hour. We see that this agrees well with several of our findings.

No one, however, had noted the enormous increase in activity immediately after mixing, and it is just this phase of the matter that is of greatest importance in practical medicine.

SUMMARY OF RESULTS

1. Rabbit serum has been found to be capable of causing as high as a thirty-fold increase in the coagulative activity of lung extract. This effect is gradually replaced by a diminution in the activity of the extract below the original as the mixture is left standing.

2. Normal human serum possesses this same power, but to a less degree, both as regards the primary activation and the later inactivation. Syphilitic serum acts exactly as does the normal serum.

3. The practical importance of this activation of tissue extracts lies in its relation to the intravenous injection of protein solutions, such as organ extracts or blood serum, which may be harmless alone but may be activated sufficiently by the patient's blood to become exceedingly toxic.

BIBLIOGRAPHY

(1) Loeb: Hofmeister's Beitr. z. Chem. Physiol. u. Path., 1905, vi, 260; Ibid., 1907, ix, 185; Zeitschr. Immunitats., 1912, xii, 189.
(2) Freund: Berl. klin. Wochenschr., 1909, xlvi, 682.
(3) Dold: Zeitschr. Immunitats., 1911, x, 53.
(4) Blaizot: Compt. rend. Soc. Biol., 1911, lxxi, 534.
(5) Gley: Compt. rend. Soc. Biol., 1911, lxxi, 584.
(6) Busson: Centr. Bakt. u. Parasitenk., 1912, lxv, 142.
(7) Roger: Compt. rend. Soc., Biol., 1912, lxxii, 191.
(8) Ichikawa: Zeitschr. Immunitats., 1913, xviii, 163.

Digitized by Google

(9) Giafami: Pathologica, v, 415, through Zentralbl. Biochem. u. Biophys., 1913, xvii, 74.
(10) Schenck: Zeitschr. Immunitats., 1914, xxii, 229.
(11) Ascoli and Izar: Münch. Med. Wochenschr., 1912, lix, 1092.
(12) Dold and Ogata: Centralbl. Bakt. u. Parasitenk., 1912, liv, 175.
(13) Cesa-Bianchi: Pathologica, 1911, iii, 69 through Chem. Abstr., vi, 2641.
(14) Mills: Journ. Biol. Chem., 1921, xlvi, 135.
(15) Hirschfeld and Klinger: Deutsch. Med. Wochenschr., 1914, xl, 1607.
(16) Fränkel and Thiele: Münch. Med. Wochenschr., 1914, lxi, 2095.

Digitized by Google

Digitized by Google

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 60 APRIL 1, 1922 No. 2

STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

XI. The Action of Cocaine and Aconitine on the Pulmonary Vagus in the Frog and in the Turtle

NATHANIEL KLEITMAN

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 28, 1921

As stated in the introduction to the first report of this series (1) our knowledge of the afferent nervous mechanism of the viscera is still very incomplete. It has not been established whether stimulation of one visceral organ will continue to elicit the usual reflex response in another organ, if the afferent connection of the latter with its center has been interrupted. This research was an attempt to produce such a condition artificially. The lung was chosen as a typical viscus because complete data on the physiology of the pulmonary vagus in the frog and the turtle were collected recently by Carlson and Luckhardt (1), (4).

It might be well to recapitulate briefly the results obtained by these investigators insofar as they have a bearing on the present problem. In the frog the pulmonary vagus carries both motor and inhibitory fibers, the latter predominating and exerting a constant tonic action on the musculature of the lung. As long as the vagi are intact the lungs are kept dilated by the inhibitory impulses coming from the medulla. Destruction of the medulla or cutting the vagi causes a more or less permanent hypertonus of the lungs. Stimulation of the peripheral end of the cut vagus inhibits the lung hypertonus during the period of stimulation. In the turtle the pulmonary vagus carries mainly motor fibers. There is little evidence of any tonic activity on the part of the pulmonary vagus, except that each series of respiratory movements is

Digitized by Google

followed by a contraction of the lungs. Cutting the vagus produces no change in the lung, but the spontaneous post-respiratory contractions are abolished. Stimulation of the peripheral end of the cut vagus causes the lung to contract.

The present report comprises the results of experiments designed to elucidate the rôle of the afferent fibers of the vagus in the reflex and tonic activity of the amphibian and reptilian lung. Mechanical and electrical stimulation of the urinary bladder in the frog will ordinarily cause a reflex contraction of the lungs. The afferent pathway of the reflex arc in this case consists of the afferent fibers leading from the bladder to the spinal cord and thence by an intermediate neuron to the medulla; the efferent pathway is the pulmonary vagus. Is it possible to elicit a reflex of this nature, if no afferent impulses from the lungs could get to the medulla? Also, is the tonic inhibitory activity of the pulmonary vagus in the frog dependent upon afferent impulses reaching the center from the lungs themselves? In the turtle, will the lung whose afferent connection with the medulla has been severed contract reflexly on the inflation of the opposite lung? Will the spontaneous contractions of the lung persist under these conditions?

The greatest difficulty in experiments of this kind is in destroying or paralyzing the afferent nerve ending, or else cutting or blocking conduction in the afferent nerve fibers coming from the lung without at the same time destroying the efferent pulmonary fibers of the vagus which would of itself make it impossible for the lung to contract reflexly. Since the efferent and afferent fibers of the vagus are intermingled, it is impossible to mechanically cut one set leaving the other intact. Therefore it was thought best to employ pharmacological means, i.e., find a drug which would block the conduction in the afferent nerve fibers of the mixed nerve trunk or poison the endings of the afferent fibers in the organ itself.

The drug that suggested itself first was cocaine. It is widely employed as a local anesthetic, and while it is essentially a general protoplasmic poison, it has been shown to exert a selective action attacking the afferent nerve endings first when injected subcutaneoulsy, and the afferent nerve fibers when injected into a mixed nerve trunk as in block anesthesia. However, this selective action on afferent nerves applies only to nerves leading to the skeletal muscles. In nerves leading to visceral structures this relationship seems to be reversed. In studying the selective action of cocaine on the fibers of the vagus, Dixon (2) found that in rabbits, dogs and cats, local application of cocaine to

Digitized by Google

the vagus trunk blocked the passage of centrifugal impulses before the centripetal. This apparent difference between the "selective" effects on skeletal and visceral nerves is not due to any peculiarity of cocaine itself (which acts as a general protoplasmic poison), but rather to the difference in structure of the nerve fibers themselves. Thus in the mammal, at least, it is evident that in the skeletal nerves the centrifugal, and in visceral nerves the centripetal fibers are more resistant to the action of cocaine.

Another interesting observation was made by Kast and Meltzer (3). These investigators found that the sense of pain normally present in the visceral organs of dogs was completely abolished by subcutaneous injection of cocaine such as made by surgeons who perform abdominal operations under local cocaine anesthesia. They ascribed this phenomenon to the absorption of cocaine into the circulation. This would indicate that the nerve endings of the visceral nerves are more sensitive to cocaine than those of the skeletal nerves.

Aconitine was also made use of. It and its allies are the only drugs which when injected intravenously will poison the endings of afferent nerves. For this reason it would be especially useful for attempting to reach the afferent nerve endings by way of the circulation.

METHODS: *Frogs*. The contractions of the lung of the frog were recorded by inserting a cannula into the tip of the lung, and connecting it with a water manometer or a sensitive tambour. The animals were always decerebrated, and prepared according to the method developed by Carlson and Luckhardt (1). For local application of drugs each lung was insulated by means of sheet rubber so that no drug could get to the neighboring viscera. Drugs were injected intravenously through a cannula inserted into the median abdominal vein. To prevent a drug injected intravenously from reaching one of the lungs used as a control the pulmonary artery on that side was ligated. In such cases methylene blue was injected at the end of the experiment, and the absence of blue in the corresponding lung was used as a test of the efficacy of the method. It may be added that as a general procedure only one vagus was used, the other being ligated and the lung on that side allowed to go into hypertonus. This was an indication that the preparation was in good physiological condition. Under certain conditions the tonic inhibitory activity of the pulmonary vagi seems to be entirely abolished, so that cutting these nerves or destroying the medulla has no effect on the lungs whatever. A large number of control experiments was necessary for this reason.

Digitized by Google

Turtles. As a rule one lung (the left) was isolated and used for recording lung contractions, the other being left in communication with the outside, through a tracheal cannula, for the purposes of respiration. As in the case of the frog the procedure of preparing the decerebrated animal for lung contractions was that of Carlson and Luckhardt (4). The lung contractions were recorded by a sensitive tambour or by a water manometer. For the local application of cocaine both vagi were isolated in the neck for a length of about 2 inches. A piece of cotton about the size of a small pea was saturated with the solution of the drug, the middle portion of the isolated nerve was insulated with a strip of sheet rubber, the cotton placed on the nerve, and the rubber tied with a thread, tight enough to express the solution on the nerve, but not so tight as to compress the nerve itself. The stimulating electrodes were then applied to the proximal or distal portion of the nerve, in either case about 1 cm. away from the place where the drug was applied. For intravenous injections the external jugular vein was cannulated. In the turtle also the presence of the normal spontaneous lung contractions depended on outside conditions. During the second half of June and the very last week of August all turtles used showed spontaneous contractions of the lungs as soon as the preparations were ready for recording. During July and the greater part of August not a single turtle could be found to exhibit this phenomenon.

Experiments with cocaine on frogs: *Intravenous injections of cocaine.* Before any injection was made the reflexes were tested. The urinary bladder and a loop of the intestine were stimulated mechanically or electrically, and a reflex response elicited from the lungs and the heart. The lungs contracted and the heart was slowed or stopped entirely. Various amounts of cocaine were then injected into the median abdominal vein, and the reflexes tested after each injection. One-tenth milligram cocaine hydrochloride in 1 cc. Ringer's when given by vein, never had any effect on the visceral reflexes tested, but it caused a slight and fleeting contraction of the lung. The same result was obtained with 0.2 mgm. cocaine (fig. 1). The injection of 0.5 mgm. cocaine in 0.5 cc. Ringer's caused a very marked contraction of the lung, with a short-lasting abolition of the lung reflexes. The effect of these doses is of very brief duration. The temporary failure to elicit a reflex may be due to the injury to the center, the efferent or or afferent terminus of the reflex arc, or to both. It will be shown presently that small doses of cocaine have no effect on the center in the medulla. Whether the afferent nerve endings in the viscera were

Digitized by Google

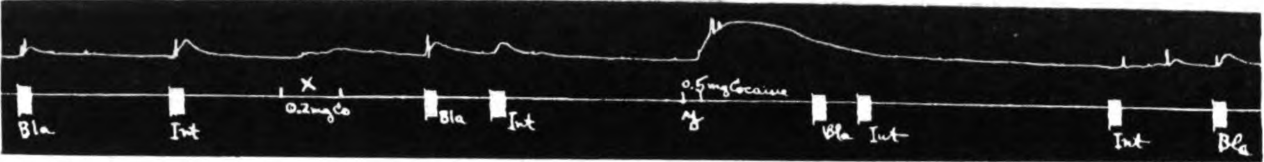


Fig. 1. Water manometer tracing of the reflex contractions of the left lung of the frog following stimulation of the bladder, *Bla*, and intestine, *Int*. Secondary coil at 10 cm., duration of stimulation 10 seconds. Effect of the intravenous injection of 0.2 mgm. x and later 0.5 mgm. y cocaine hydrochloride on the lung musculature and the lung reflexes. Showing temporary hypertonus of the lung and abolition of reflexes into the lung following intravenous injection of cocaine with return of the normal inhibitory tonus of the lung and lung reflexes as the action of the drug wore off.

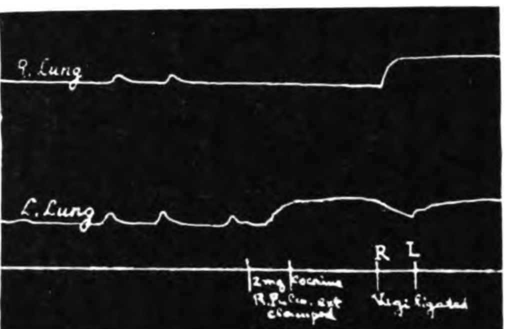


Fig. 2. Upper tracing is a tambour record of the intrapulmonic pressure of the right lung of a frog; lower tracing is a water manometer record of the pressure in the left lung. Right pulmonary artery clamped, and 2 mgm. cocaine injected into the median abdominal vein. R = right vagus ligated; L = left vagus ligated. Showing that the hypertonus of the lung following the intravenous injection of moderate doses of cocaine is due to a peripheral and not a central action of the drug.

Digitized by Google

poisoned could not be determined. Stimulation of the mesentery which contained nerve fibers (never affected by a poison in circulation) likewise failed to produce a reflex contraction of the lung. This would indicate that the efferent inhibitory fibers of the vagus were temporarily paralyzed or depressed. The contraction of the lung which becomes more marked as the dose of cocaine is increased, and eventually simulates the effect produced by cutting the vagus, confirms this supposition. It may be added that the inhibitory endings of the vagus in the lung showed approximately the same resistance to the action of cocaine as the inhibitory endings in the heart. Sometimes after the injection of 0.5 mgm. cocaine the heart reflexes disappeared while those of the lung persisted, and sometimes the opposite was true. The injection of 1 mgm. cocaine or more abolished both the lung and the heart reflexes invariably, and for a long time. In either case the efferent vagus terminations were paralyzed. In the lung cocaine produced the characteristic hypertonus that ensues on ligation of the vagus. That the so-called "escape" of the lung from the inhibitory control of the vagus was of purely peripheral origin was shown by the following experiment (fig. 2). The right pulmonary artery was clamped off, and the blood vessels in the right lung gradually disappeared. Two milligrams of cocaine were then injected intravenously. The left lung went into a condition of hypertonus, but the right lung was not affected, thus showing that the center in the medulla was not paralyzed. Ligation of the right vagus caused the usual "escape" of the right lung, confirming the fact that the center continued to send tonic inhibitory impulses after the injection of cocaine. In the meantime the effect of cocaine on the left lung was beginning to wear off, and the lung was gradually dilating. At this point ligation of the left vagus caused the lung to contract again, but it will be noticed this contraction was of the same magnitude as that caused by the cocaine. This shows that the previous contraction was a true "escape" from vagus inhibition caused by the paralysis of the inhibitory endings of the vagus in the lung.

Stimulation of the vagus after the intravenous injection of cocaine produced no relaxation of the contracted lung, if the induced current was not very strong. With very strong currents inhibition of the lung could be obtained even after 2 mgm. cocaine were injected. With larger doses of cocaine (5 to 8 mgm.) even the strongest current produced no dilatation of the lung, nor slowing of the heart. This would indicate that the efferent inhibitory endings were not actually paralyzed by the

Digitized by Google

smaller doses of cocaine, but only depressed so that the ordinary tonic inhibitory impulses from the medulla could not reach the lungs (thus producing hypertonus). Strong stimuli, however, could "break through" the depression, and so produced dilatation of the lung.

Intravenous injection of cocaine after the ligation of the vagi was frequently without any further effect. Occasionally large doses of cocaine produced a further contraction, apparently by direct action on the peripheral mechanism.

In several experiments the skeletal reflexes were tested along with the visceral. The central end of the cut sciatic nerve of one leg was stimulated with a very weak induced current, and it was noticed that even large amounts of cocaine did not abolish the contralateral reflexes. If anything, the skeletal reflex excitability was increased, a well-known effect of cocaine. Thus, it is evident that the efferent endings of the skeletal nerves are much more resistant to the action of cocaine than the efferent endings of visceral nerves.

Intra-arterial injections of cocaine. It was interesting to determine the dose of cocaine which when injected intravenously would cause an "escape" of the lungs from vagus inhibition by paralyzing the center in the medulla. It will be recalled that 1 to 2 mgm. cocaine produce this effect by purely peripheral action. Prior to injecting the drug both pulmonary arteries were clamped or ligated, to prevent the cocaine from getting to the endings of the vagus in the lungs. As larger amounts of cocaine were injected the effect on the heart interfered with the experiments. The heart would stop, and the drug would get to the lungs by way of the pulmonary veins. Under these circumstances the lungs contracted, but this was due to the peripheral effect of the drug, and the effect on the center, if any, was masked. That this was the case was shown by the fact that methylene blue injected intravenously, with the heart at standstill, found its way to the lungs, in spite of the ligation of the pulmonary arteries.

It may be pertinent to record some observations on the action of cocaine on the heart in situ. Small amounts (1 to 2 mgm.) injected intravenously merely slowed the heart temporarily. Larger doses or repeated injections of small doses often produced complete arrest of cardiac action, the ventricle being enormously dilated and engorged with blood. In some cases complete heart block was observed; this gradually changed into 6:1 rhythm, then to 5:1 rhythm, and so on, until the heart recovered completely. So far as I am aware, this effect was never reported before. However, large amounts of cocaine could

Digitized by Google

be injected very slowly without causing the heart to stop even temporarily. Thus into one frog 7 mgm. cocaine were injected, and forty minutes later another injection of 7 mgm. was made, without causing the heart to stop. In each case the heart was slowed considerably, and "peristaltic" movements of the ventricle were observed.

This action of cocaine on the heart made it impossible to paralyze the vagus center by intravenous injections. At the suggestion of Doctor Luckhardt, the following procedure was employed with success. Both pulmonary arteries were ligated. A cannula was inserted into the left aortic branch, the mouth of the cannula being directed toward the heart. The cocaine injected through this cannula was driven first toward the heart, but at the bifurcation of the aorta it mixed with blood and was carried to the medulla. This could actually be seen through the walls of the vessels, and if the injection was made slowly, no cocaine would get into the heart. Apparently no excessive pressure was developed in the aorta, and the effect on the medulla could not have been mechanical.

By means of this method various amounts of cocaine had to be injected to paralyze the vagus center, the average dose being 5 mgm. As the drug was given unilaterally this dose corresponds to 10 mgm. cocaine given intravenously. Subsequent injection of methylene blue gradually caused the entire body of the frog, the lungs excepted, to be colored with the dye, thus confirming the fact that the action was central.

It will be noted that a dose of cocaine sufficient to paralyze the endings of the vagus in the lungs will not paralyze the center in the medulla showing that the cells in this case are more resistant to the action of cocaine circulating in the blood than the axone terminations. This fact is not in agreement with the general conception regarding the action of cocaine. Thus Cushny in his *Text-book of Pharmacology* (5), speaking of cocaine, says that "if brought in contact with other forms of living matter in the concentration used in anesthetizing nerve ends, it is poisonous to all structures which have been examined. Even concentrations too low to act on the peripheral nerves act on the nerve cells and paralyze them. . . . ." It must also be remembered that selective action of a general protoplasmic poison merely indicates a difference in structure in the various forms of protoplasm acted upon. It must be concluded, therefore, that the efferent endings of the visceral nerves differ greatly from the endings of skeletal nerves in their resistance to the action of cocaine. This conclusion, if correct, would

Digitized by Google

Fig. 3. Water manometer record of the intrapulmonic pressure of the right lung of a frog. *I* = 1 mgm. cocaine in ½ cc. Ringer's applied to the surface of the lung producing a marked contraction. The lung relaxed when the cocaine was washed off. *II:* A second application of 1½ mgm. cocaine produced a similar effect. Subsequent ligation of the vagus at *R.V.*—no further contraction. Showing the prompt but temporary hypertonus of the lung is due to a depression or paralysis of the inhibitory nerve endings of the vagus in the lung when cocaine is applied locally to the lung in moderate doses.

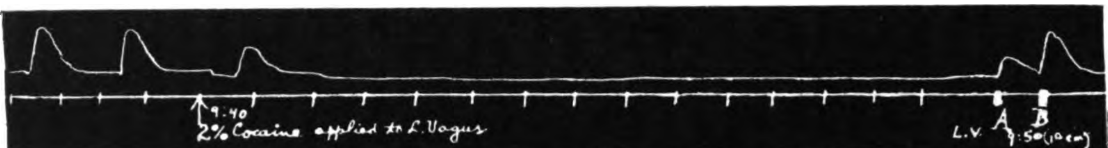


Fig. 4. Water manometer tracing of the spontaneous contractions of the turtle's lung. Signal marks indicate respiratory movements. Application of a 2 per cent cocaine solution to the vagus trunk abolished spontaneous contractions of the lung. Respiration continued. Stimulation of the vagus above, *A*, and below, *B*, the point where the drug was applied produced contraction of the lung.

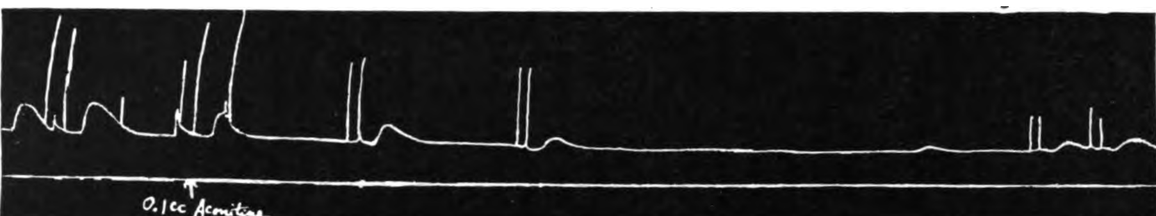


Fig. 5. Water manometer tracing of the intrapulmonic pressure of the turtle's lung. Five minutes after the intravenous injection of 0.1 cc. of a saturated solution of aconitine, respiration stopped and the spontaneous lung contractions disappeared at the same time. Eight minutes later both respiration and spontaneous lung contractions returned.

explain the results obtained by Kast and Meltzer (3) who found that in local cocaine infiltration anesthesia of the abdominal area pain sensations from the viscera are entirely abolished. They held that some cocaine was absorbed into the circulation, and that this small amount of cocaine paralyzed the endings of the pain fibers in the viscera, while it was unable to produce general body anesthesia. In the light of the above-mentioned facts such an explanation seems to be very plausible, as it is based on the assumption that the visceral nerve endings offer a very low resistance to the action of cocaine.

Local application of cocaine. When 0.1 mgm. cocaine in 1 cc. Ringer's is applied to the surface of the frog's lung, a slight and transient contraction is produced. With larger amounts of cocaine the contractions are more marked and more lasting. One milligram cocaine in 1 cc. Ringer's will produce a maximal contraction of the lung (fig. 3). If the cocaine is washed off the lung will relax and gradually return to its original size. A second application of an equal amount of cocaine will produce precisely the same effect, and this can be repeated many times. If the vagus is ligated shortly after the application of cocaine (fig. 3) no further contraction is produced showing that the cocaine paralyzed the nerve endings and that the contraction was maximal. Stimulation of the peripheral part of the ligated vagus will not cause the lung to relax, unless the current is very strong. If the cocaine applied to the lung is not washed off, the lung will relax nevertheless, but only after a considerable interval of time. This is apparently due to the absorption of the cocaine into the circulation, as in this case a second application of cocaine or ligation of the corresponding vagus will produce a maximal contraction of the lung. To confirm the conclusion that local application of cocaine will temporarily depress or even paralyze the efferent inhibitory endings of the pulmonary vagus use was made of the nicotine method. Carlson and Luckhardt (1) have shown that after the vagus in the frog was ligated, and the corresponding lung "escaped" from its tonic inhibitory control, the injection of nicotine effected a marked dilatation of the lung by stimulating the efferent inhibitory endings of the pulmonary vagus. The following experiment was performed. The right vagus was ligated producing hypertonus of the right lung; 2 mgm. cocaine (1 cc.) were applied to the left lung producing a similar hypertonus, and subsequent ligation of the left vagus had no further effect. One milligram nicotine in 1 cc. Ringer's was now injected into the median abdominal vein, and this produced an immediate inhibition of the right lung, the left lung remaining unaffected.

Digitized by Google

When a 2 per cent solution of cocaine was applied to the vagus directly, the corresponding lung contracted markedly. Subsequent ligation of the vagus produced no further contration of the lung; nor did stimulation of the vagus above the point of application of the drug cause inhibition of the lung, unless the current was very strong. This shows that local application of cocaine will abolish conductivity in the fibers of the vagus.

EXPERIMENTS WITH COCAINE ON TURTLES: *Intravenous injections of cocaine.* As little as 0.5 mgm. cocaine hydrochloride in 1 cc. Ringer's, when injected into the external jugular vein, may abolish the spontaneous post-expiratory contractions of the turtle's lung for one hour. This, however, is a rare occurrence. Ordinarily 3, 4 or 5 mgm. cocaine must be injected to abolish these contractions for a like period of time. The length of time required for complete recovery was not exactly proportional to the dose given, but depended also upon the physiological condition of the preparation. The lung reflexes were abolished together with the spontaneous contractions, and returned with them. Stimulation of the vagus produced no contraction of the lung, unless the current was very strong. It will be noticed that the effect of cocaine injected intravenously on the endings of the motor fibers of the vagus in the lung of the turtle is essentially the same as that on the endings of the inhibitory fibers of the vagus in the lung of the frog. The skeletal reflexes were not suppressed even by the largest doses of cocaine (up to 20 mgm.), showing that, as in the frog, the efferent endings of the skeletal nerves are more resistant to the action of cocaine than the efferent endings of the vagus.

The heart could not be observed directly, but the pulse in the arteries on the surface of the lung served as a criterion of the condition of the ventricle. In the doses used cocaine slowed the pulse temporarily. In one turtle the pulse disappeared for fifteen minutes after the injection of 8 mgm. cocaine.

Application of cocaine to the trunk of the vagus. Weak solutions of cocaine (0.5 per cent) applied to the vagus in the neck have no effect on the conductivity of its fibers. The spontaneous contractions of the lungs are not abolished. Stronger solutions of cocaine, 2 to 5 per cent, will temporarily abolish the spontaneous contractions, an effect usually following the division of the vagus. This would indicate that cocaine blocked the conduction in the efferent fibers of the vagus. To test this, the vagus was stimulated electrically centrally and peripherally to the point where the cocaine was applied. While stimulation below

Digitized by Google

that point always produced a very marked contraction of the lung, stimulation above it produced no contraction, or a feeble contraction, when the concentration of the cocaine solution was low, and the current very strong. Upon the removal of the drug and repeated washing of the nerve with Ringer's solution the conductivity of the fibers gradually returned. Stimulation of the vagus above the point where the cocaine was applied produced stronger and stronger contractions of the lung, although the strength of the current and the duration of stimulation remained constant. The spontaneous contractions of the lung returned also. The degree of depression was greater the higher the concentration of the cocaine solution applied. The adherents of the "all-or-none" theory as regards the conduction of nerve impulses would say that the record shown in figure 4, where stimulation above the point of application of cocaine produced a feebler contraction of the lung than stimulation below that point, merely indicates that only the outside fibers of the vagus were affected by the 2 per cent cocaine solution, and contraction *A* was a result of stimulation of a fewer number of fibers (the inside fibers not affected by cocaine). This explanation would make it unnecessary to assume that there was a depressed conductivity in all the fibers. But it would be difficult to explain why the normal impulse following each series of respiratory movements failed to reach the lung and produce a contraction. Obviously, if the inside fibers were unaffected, some contraction of the lung, though possibly a very feeble one, should follow the respiratory movements. Since the spontaneous contractions disappeared entirely, it seems more probable that cocaine *depressed the conductivity in all the fibers* of the vagus. Apparently the normal periodic stimuli sent along the vagus from the lung-motor center were blocked by the depressed conductivity of the fibers of the vagus. A weak electrical stimulus was also blocked, while a strong electrical stimulus would pass in spite of the depressed state of the fibers. And that is what was actually observed.

Experiments were also performed to detect a difference, if any, in the effects produced by the local application of cocaine on the efferent and afferent fibers of the vagus. While the results obtained were not uniform, it may be stated that the afferent fibers are more resistant to the action of the drug than the efferent fibers. The procedure employed was as follows. The left lung was connected with a water manometer. Both vagi were isolated. Stimulation of the right vagus produced a reflex contraction of the left lung. A 5 per cent solution of cocaine was applied to the right vagus, and the nerve stimulated cen-

Digitized by Google

trally and peripherally to the point where the drug was applied. In about 30 minutes conduction in the afferent fibers was blocked, and stimulation of the right vagus peripherally to the point of application of cocaine failed to elicit a reflex contraction of the left lung. Washing the cocaine off the vagus would sometimes restore the conductivity of its afferent fibers, but only after several hours. As a rule, cocaine was applied next to the left vagus of the same turtle. The conductivity of the efferent fibers was abolished in less than ten minutes, and stimulation of the vagus centrally to the point of application of cocaine produced no contraction of the lung. Washing the cocaine off the left vagus ordinarily restored the conductivity of its efferent fibers in an hour or two, and in one case in about ten minutes. It is evident that the efferent fibers of the vagus are much less resistant to the action of cocaine than the afferent fibers.

Experiments with aconitine on frogs: According to the U. S. Pharmacopeia, aconitine is almost insoluble in water. For use in these experiments a few crystals of the drug were shaken up with a large quantity of Ringer's solution, and after the crystals settled on the bottom the supernatant liquid was poured off and used as a saturated solution of aconitine. It must be borne in mind that on account of its extreme insolubility a "saturated" solution of aconitine may be likened to a saturated solution of silver chloride or barium sulphate; in other words, though saturated, it is still extremely dilute. No attempt was made to determine the exact concentration of the solution, as the drug very often contains impurities, aconine and benzaconine, and its activity varies considerably. Some samples are one hundred times stronger than others. To assay roughly the potency of the aconitine used, various amounts of the saturated solution were injected into frogs. It was found that 0.3 cc. of aconitine injected into the ventral lymph sac will paralyze a frog weighing 35 grams in about forty minutes, and kill it in one and a half hours. Fibrillary twitching of the skeletal muscles and strychnine-like convulsions preceded the paralysis. The motor endings of the skeletal nerves preserve their irritability, as stimulation of the sciatic nerve produced a contraction of the gastrocnemius.

Intravenous injections of aconitine. When injected intravenously, $\frac{1}{20}$ or even $\frac{1}{50}$ cc. of the saturated solution of aconitine will cause an "escape" of the lungs from vagus inhibition, by paralyzing the efferent inhibitory endings of the pulmonary vagus. In order to show that this action was local, the same method was used as in experiments with cocaine. The right pulmonary artery was clamped, and the injection of aconitine produced no contraction of the right lung,

Digitized by Google

although the left lung contracted immediately. Subsequent ligation of the right vagus produced an escape of the right lung from vagus inhibition, showing that the center continued to exert its tonic inhibitory activity after the injection of aconitine.

Intra-arterial injections of aconitine. To determine the dose of aconitine that would produce a contraction of the lungs by paralyzing the vagus center in the medulla larger quantities of the drug had to be injected, and its action on the heart, such as reversal of rhythm, made it necessary to use intra-arterial injections. The procedure and control were the same as employed in the intra-arterial injections of cocaine. It was found that 0.1 cc. of the saturated solution of aconitine when administered in this way will paralyze the vagus center and cause the lungs to contract. This dose corresponds to an intravenous dose of 0.2 cc.

Local application of aconitine. Variable results were obtained with this method. However, the dose necessary to paralyze the endings of the vagus by local application was very large. Sometimes 1 cc. of the saturated solution (three times the lethal dose when injected into the ventral lymph sac) had to be applied to the lung to produce an "escape" from vagus inhibition. Very often peculiar spontaneous rhythmic contractions of the lungs would appear after the local application of aconitine, and each lung showed a rhythm of its own. It is possible that aconitine stimulates the local nervous mechanism, while paralyzing the inhibitory endings of the vagus.

Experiments with aconitine on turtles: The intravenous injections of very small amounts of aconitine were without effect. But when the dose was increased to 0.1 cc. of the saturated solution, aconitine abolished the respiratory movements, and the spontaneous lung contractions that follow them, in about five minutes (fig. 5). Both respirations and lung contractions returned in eight minutes; they were feeble at first, but soon regained their original magnitude. The intravenous injection of 0.2 cc. aconitine abolishes respiratory movements and spontaneous lung contractions for four or five hours, but when respiration was restored, the lung contractions were invariably restored with it. Stimulation of the vagus before and after the injection of aconitine produced lung contractions of exactly the same magnitude, showing that the efferent nerve endings were not paralyzed. Permanent cessation of respiration and disappearance of spontaneous lung contractions were observed after the injection of 0.5 cc. of aconitine. The action was central, as similar results were obtained when the pulmonary artery was ligated. Moreover, with the lung circulation intact, stimu-

Digitized by Google

lation of the vagus after the injection of 0.5 cc. aconitine produced a contraction of the lung. Stimulation of the opposite vagus also produced a contraction of the lung (reflex) indicating that the lung-motor center was intact. The only conclusion possible is that the respiratory center was paralyzed by aconitine. A very characteristic phenomenon was the contracture of the lung after the injection of aconitine (fig. 6). Subsequent stimulation of the vagus on the same or opposite side produced an added contraction of the lung, without relaxation. In some experiments this contracture did not occur spontaneously, but only after one of the vagi was stimulated.

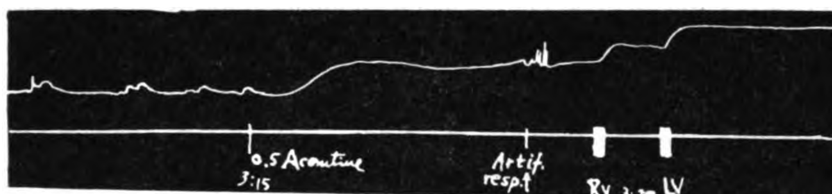


Fig. 6. Tambour record of the intrapulmonic pressure of the left lung of the turtle. Intravenous injection of 0.5 cc. of aconitine abolished respiration and spontaneous lung contractions. The lung went into a state of contracture. Stimulation of the right and left vagus produced further contractions of the lung, but no relaxation followed these contractions. Drum moving very slowly.

The pulse in the lung arteries was weakened and slowed by the intravenous administration of aconitine.

The lethal dose of aconitine for the turtle varied between 2 and 5 cc. of the saturated solution.

SUMMARY

1. The intravenous injection of 1 mgm. cocaine hydrochloride will abolish the lung and heart reflexes in the frog by paralyzing or greatly depressing the efferent endings of the vagus. The lung "escapes" from the inhibitory control of the vagus for the same reason. Whether the afferent endings are similarly affected has not been established.

2. A dose of cocaine sufficient to paralyze the efferent endings of the vagus is without effect on the efferent endings of the skeletal nerves.

3. A much larger dose of cocaine is necessary to produce an "escape" of the lungs by paralyzing the vagus center. As a general protoplasmic poison, cocaine is held to be more poisonous to the cells of the central nervous system than to peripheral nerve endings, but in the vagus, at least, the cells are more resistant to the action of the drug than the efferent nerve endings.

Digitized by Google

4. Intravenous injection of cocaine will produce slowing of the heart, if the dose is small (1 to 2 mgm). Larger doses cause complete or partial heart block, and sometimes "peristalsis" of the ventricle.

5. Local application of cocaine to the lung of the frog has the same effect as the intravenous injections of the drug.

6. Intravenous injection of cocaine will abolish the spontaneous lung contractions in the turtle by paralyzing or depressing the efferent motor nerve endings of the pulmonary vagus, essentially the same effect as that observed in the frog.

7. The spontaneous lung contractions in the turtle disappear after a 2 to 5 per cent cocaine solution had been applied directly to the vagus trunk in the neck. Apparently the efferent impulses from the lung-motor center are blocked by the cocaine. Stimulation of the vagus above the point of the application of the drug produces a feebler contraction of the lung than stimulation below that point. This is not due to blocking the conduction in some fibers, but rather to a depression of the conductivity in all the fibers.

8. The afferent fibers of the pulmonary vagus are more resistant to the action of cocaine than the efferent fibers. A similar result was obtained by Dixon on the vagus of the dog, the cat and the rabbit.

9. Minute doses of aconitine will paralyze the efferent inhibitory endings of the pulmonary vagus in frogs, and larger doses will paralyze the vagus center. This resembles the action of cocaine on the same structures.

10. Intravenous injection of aconitine will paralyze the respiratory center in turtles, and thus indirectly abolish the spontaneous contractions of the lungs. Aconitine also produces a state of contracture in the turtle's lung.

It is a pleasure to acknowledge my indebtedness to Dr. A. J. Carlson for suggesting this study, and to Dr. A. B. Luckhardt for his constant aid in overcoming the technical difficulties and for his numerous suggestions as to the control of the experiments.

BIBLIOGRAPHY

(1) Carlson and Luckhardt: This Journal, 1920, liv, 55.
(2) Dixon: Journ. Physiol., 1904, xxxii, 87.
(3) Kast and Meltzer: Med. Rec., December 29, 1906.
(4) Carlson and Luckhardt: This Journal, 1920, liv, 261.
(5) Cushny: A text-book of pharmacology and therapeutics, Philadelphia and New York, 1918, 359.

Digitized by Google

STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

XII. The Response of the Isolated Esophagus of the Frog and the Turtle to Certain Drugs

Z. BERCOVITZ

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 28, 1921

It was reported by Carlson and Luckhardt (1) that in the turtle stimulation of the peripheral vagus causes inhibition of the circular neuro-muscular mechanism of the upper two-thirds of the esophagus and contraction of the longitudinal system in the region of the cardia. This would seem to indicate a difference in innervation of the esophagus at different levels. These investigators also noted that the hypertonus of the frog's esophagus following vagi section and vagi stimulation seemed to be confined principally to the longitudinal system.

The present study was undertaken at the suggestion of Doctors Carlson and Luckhardt with the view of ascertaining whether further light on the motor control of the esophagus might be secured by the study of the reaction of isolated preparations of the esophagus to the so-called neurotropic and myotropic drugs.

Methods: The esophagus was prepared for studying the circular and longitudinal neuro-muscular systems in the following manner:

1. Frog. In the experiments on the frog the entire esophagus was used. In studying the circular system the esophagus was suspended as a ring in a muscle warmer and connected with German silver wire hooks to a delicate heart lever which recorded on a slowly moving drum. After the frogs were pithed and the esophagus exposed usually less than one-half a minute was required to cut it off at the cardia and pharynx and suspend it in the bath.

It was found necessary to turn the esophagus inside out before any dependable reactions to the drugs could be obtained. With the mucous membrane in its normal position, that is, inside, the circular muscle fibers and their nervous mechanism seem to be almost completely inaccessible to the drugs in solution in the bath.

Digitized by Google

In the study of the longitudinal neuro-muscular mechanism the whole esophagus again was used. One end was attached by means of a small clamp to a fixed point in the muscle warmer and the other by a similar clamp by a silk thread to a delicate heart lever. The weight of the clamps was carefully counterbalanced.

The fluid in the muscle warmer was changed by displacing it from below, thus preventing exposure of the tissue to the air.

2. *Turtle.* After pithing the turtle and removing the plastron the entire esophagus and stomach were carefully cut away from their surrounding tissues. In studying the circular system two methods of suspending the preparations were used. First, the same method as used in studying the circular fibers of the frog esophagus. Another method was to use the small clamps. These were applied to the esophagus so that the direction of work of the muscle was the same as when it was used as a ring. The whole ring was used in this case also.

The clamps were used entirely when studying the longitudinal system, strips of about 1 cm. in length being used.

In all the work on turtle tissues two or three rings or strips were usually suspended in the same bath. This was accomplished by using a piece of glass rod for a holder and having the clamps or hooks attached to it. This was dipped into a container made from an inverted bottle from which the bottom had been cut away. In this case the fluid was drained from the bottom of the container and the fluid to replace it was then poured in promptly. The tissues in this case were thus exposed to the air for about five seconds at each change of the bath. This was carefully checked by repeated changes with the Ringer's solution. It was found that no appreciable changes followed this short exposure to the air. In all the work the fluids were used at room temperature.

RESULTS ON THE FROG ESOPHAGUS: 1. *Need of oxygen for the spontaneous activity of the circular mechanism.* Early in the work it was noted that when the preparation was immersed in a non-oxygenated Ringer's solution there was an inhibition of the spontaneous contractions and the tone of the isolated ring of esophagus (see fig. 1, *C*).[1] This inhibition did not occur if oxygen had been previously bubbled through the Ringer's solution (fig. 1, *C*).

When the ring of the esophagus is isolated and suspended in oxygenated Ringer's solution only a few minutes are as a rule required before the development of a vigorous rhythm. If the ring be suspended

[1] The tracings in this article are reduced about two-thirds.

Digitized by Google

in non-oxygenated Ringer's solution there may or may not be a few spontaneous contractions followed by hypertonus (see fig. 1, *A*). Addition of oxygen to the bath with the preparation in this condition is followed by an increase in tone and a vigorous spontaneous activity in the ring (fig. 1, *A*). If in an active preparation the oxygen be shut off and non-oxygenated Ringer's solution be placed in the bath there is inhibition of the contractions and tone and finally complete cessation

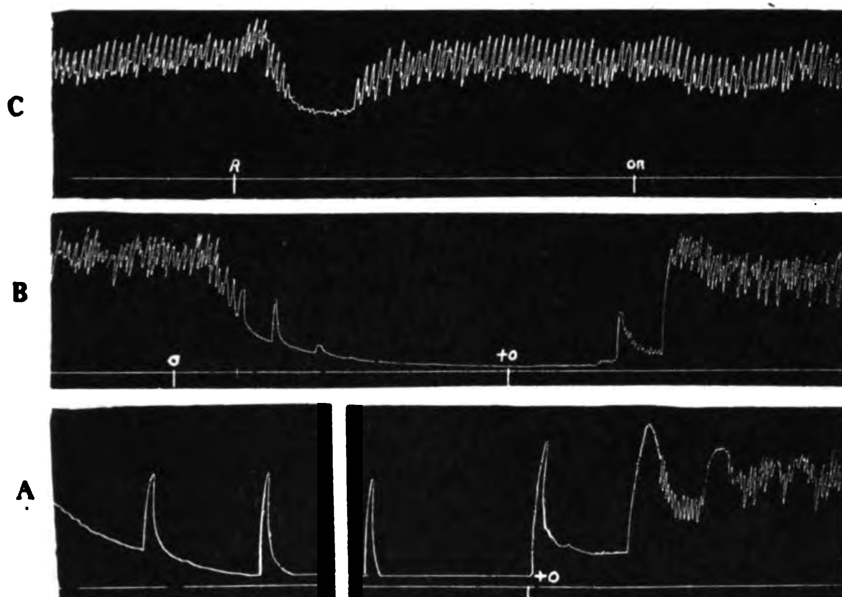


Fig. 1. Frog esophagus. Record of isolated circular neuro-muscular system. A; suspended in Ringer's solution without oxygen; showing complete hypotonus: +*O*, start bubbling oxygen through bath, showing increased tone and development of spontaneous rhythm. B; active spontaneous contractions in oxygenated Ringer's solution; —*O*, shut off oxygen and replaced bath with non-oxygenated Ringer's solution; showing decrease in tone and failure of contractions: +*O*, replaced oxygen, showing recovery of tone and contractions. C; active spontaneous contractions in oxygenated Ringer's solution. *R*, changed bath with non-oxygenated Ringer's solution; showing temporary inhibition. *OR*, changed bath with oxygenated Ringer's solution and showing no effects on the preparation.

of all spontaneous activity (fig. 1, *B*). With the ring in this condition the addition of oxygen to the solution is again promptly followed by spontaneous contractions and increased tone to the previous level (fig. 1, *B*). *This indicates that oxygen is necessary for the spontaneous activity of the isolated ring of frog's esophagus.*

Digitized by Google

The inhibition in non-oxygenated Ringer's solution was not as great in preparations which had been previously inverted. In view of this influence of non-oxygenated Ringer's solution on the spontaneous activity of the isolated ring, in all subsequent work on this type of preparation the precaution was taken of using only oxygenated Ringer's solution in making up the dilutions of the drugs to be studied. The longitudinal mechanism does not seem to be as sensitive as the circular system to changes in the oxygen supply.

When the isolated ring of esophagus is suspended in a bath through which air is bubbled instead of oxygen, a poor and irregular spontaneous activity develops for a short time. The tone is low and the contractions are far apart with an early failure. If at this time oxygen be substituted for the air there is a prompt increase in tone and the development of a vigorous rhythm. In the active preparation replacing oxygen with air or changing the bath to a non-oxygenated Ringer's solution is soon followed by complete failure of the contractions and a drop in tone. The substitution of oxygen for air or for non-oxygenated Ringer's solution is followed by a restoration of tone and contractions. As a rule no inhibition occurs if the bath be changed with Ringer's solution through which air has been bubbled for a long time (one hour or more). If in the active preparation the bath be changed with aerated Ringer's solution and air be substituted for oxygen there is a cessation of the spontaneous contractions and complete inhibition of tone.

2. Adrenalin. In 14 experiments there was an increased tone of the circular ring following the administration of adrenalin in a dilution of 1:100,000 (fig. 2, *A*). This increase was prompt in its occurrence and always marked. The recovery was slow.

In 7 experiments an abrupt and profound inhibition of both tone and contractions followed adminstration of the same quantity of adrenalin (fig. 2 *B*). Whichever reaction was obtained it could always be repeated on the same preparation. It was never possible on the same preparation to obtain stimulating and also inhibiting effects with adrenalin. Both adrenalin reactions were obtained after atropine.

Addition of adrenalin to the bath was always followed by an inhibition of the longitudinal system (fig. 3, *A* and *B*). This inhibition consisted of both a drop in tone and also cessation of the spontaneous contractions. This inhibition always came on promptly and usually remained until the drug was removed from the bath. Recovery was also prompt. The inhibitory effect of adrenalin is more marked after atropine than before, in the same preparation (fig. 3, *B*). Repeated

Digitized by Google

administrations of adrenalin in the same preparation are followed by decreased reactions.

It was found that a definite inhibition of the longitudinal system could usually be obtained with adrenalin in dilutions up to 1:30,000,000. In some sensitive preparations the reaction was obtained with a dilution of adrenalin of 1:100,000,000 (fig. 3, *A*). The influence of atropine on the reaction to this dilution of the drug was not tested out.

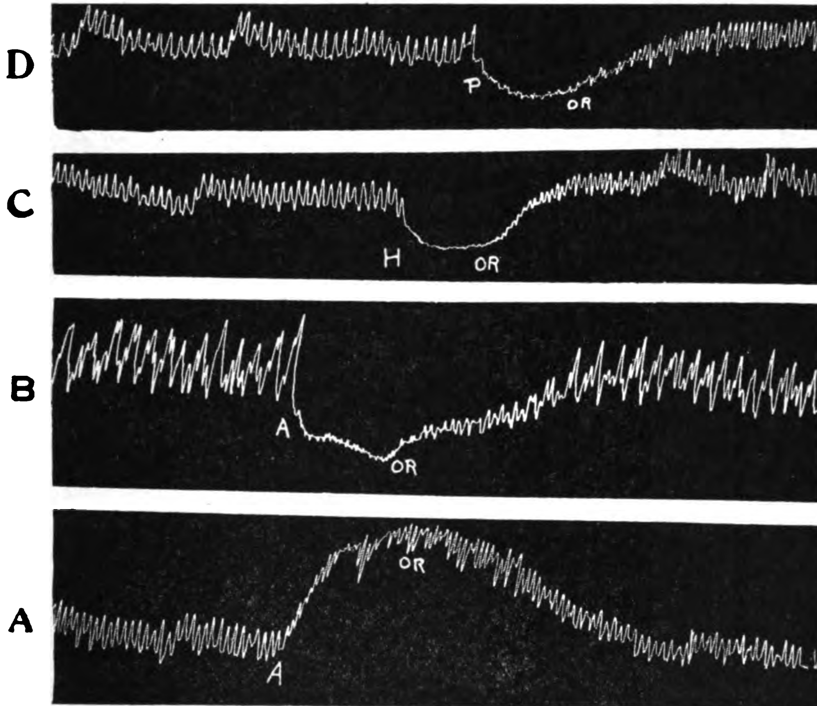


Fig. 2. Frog esophagus. Record of isolated circular neuro-muscular system in oxygenated Ringer's solution. *OR,* changed bath with oxygenated Ringer's solution. A: showing the stimulating influence of adrenalin, *A*; B: the inhibition in another preparation from the same quantity of adrenalin (1:100,000); C; the inhibitory influence of histamine, *H*; D: the inhibition after pituitary liquid, *P*.

3. Pilocarpine. Stimulating effects always follow the administration of pilocarpine to preparations of the frog's esophagus in dilutions of 1:100,000. These are the same for both circular and longitudinal systems and include a prompt and abrupt rise in tone. After a short

Digitized by Google

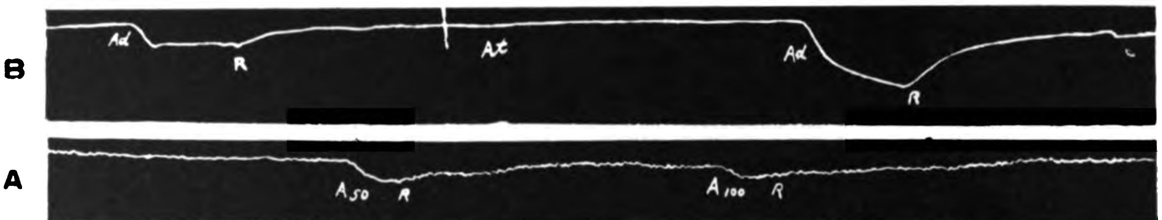


Fig. 3. Frog esophagus. Record of longitudinal neuro-muscular system in oxygenated Ringer's solution. A: showing inhibition caused by adrenalin in dilutions of 1:50,000,000, *A–50*, and 1:100,000,000, *A–100*. B: showing inhibition caused by adrenalin, *Ad*, in dilution of 1:100,000 before and after atropine, *At*.

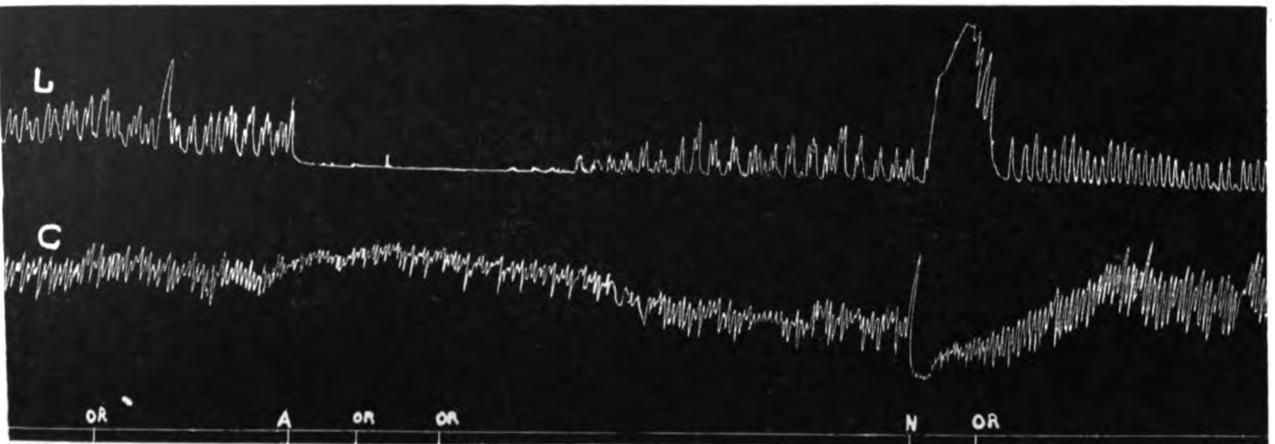


Fig. 4. Frog esophagus. Simultaneous records of circular system, *C*, and longitudinal system, *L*. *OR*, washed with oxygenated Ringer's solution. *A*, adrenalin (1:100,000) showing stimulation of circular system and inhibition of longitudinal system. *N*, nicotine (1:1,000,000) showing inhibition of circular system and stimulation of longitudinal system.

Digitized by Google

period of time there may be the development of a vigorous rhythm at the higher tone level. Recovery from this reaction is usually slow and requires several changes with oxygenated Ringer's solution.

4. Nicotine. Nicotine in dilutions of 1:100,000 acts essentially the same for both circular and longitudinal systems although the predominating action is different. When nicotine is applied to a preparation there is a prompt inhibition of the tone. The inhibition is more marked in the circular system than in the longitudinal system and the latter, if the tone is low, may be missed entirely (fig. 4). Following this inhibition there is a stimulating phase of nicotine which is more pronounced in the longitudinal system than in the circular system (fig. 4). In the circular system the stimulation is more of a tonus change while in the longitudinal system it appears as a sharp contraction and a rapid recovery. The nicotine effects could be obtained several times on the same preparation.

5. Atropine. If atropine be administered in dilutions of 1:100,000 after pilocarpine thêre follows a decrease in tone and also inhibition of the contractions which may have been initiated by the latter drug. This inhibition is uaually permanent and occurs in both circular and longitudinal systems. As a rule atropine is without effect on the circular system when under stimulation of pilocarpine.

In the case of the longitudinal system atropine is followed by a slight temporary inhibition. This reaction occurs with marked regularity.

6. Histamine and pituitrin. The addition of histamine hydrochloride in dilutions of 1:100,000 to preparations of frog's esophagus is followed by a prompt and abrupt inhibition of both the tone and the contractions (fig. 2, *C*). There is prompt recovery on removing the drug from the bath. The action of histamine is the same on both the circular and longitudinal systems.

There is a striking similarity between the action of histamine and that of adrenalin on the longitudinal system. On the circular system histamine inhibition is more constant than the adrenalin inhibition. The histamine reaction like that of adrenalin occurs after atropine. There is no instance in this series in which histamine was followed by a stimulating effect. This seems to indicate that the histamine action on this organ is nervous rather than muscular, a view previously expressed by Luckhardt and Carlson (2) in their work with this drug on the salamander lung. This is not in accord with the generally accepted conception of histamine as a direct stimulant of smooth muscle.

Digitized by Google

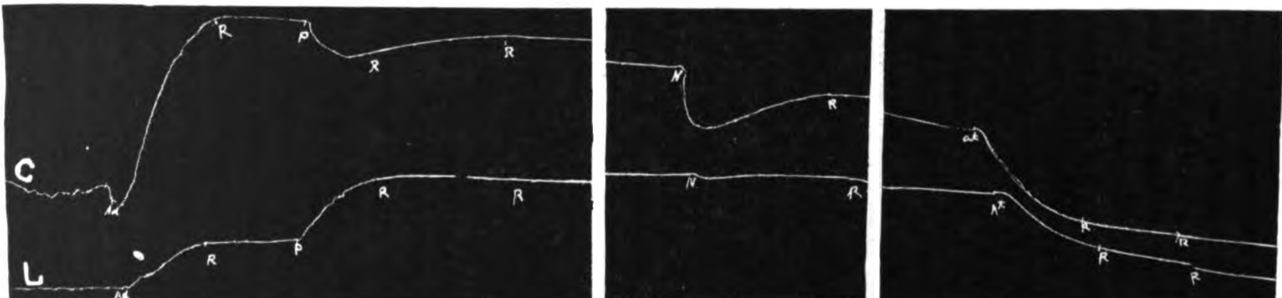


Fig. 5. Turtle esophagus. Stimultaneous record of circular system, *C*, and longitudinal system, *L*. *Ad*, adrenalin (1:200,000) showing greater stimulation on circular system than longitudinal system. *P*, pilocarpine (1:100,000) showing further stimulation of longitudinal system and only inhibition of circular system. *R*, wash with Ringer's solution. *N*, nicotine (1:100,000) showing inhibition of circular system and no action on longitudinal system. *At*, atropine (1:100,000) showing inhibition of both circular and longitudinal systems.

The frog's esophagus is described (3) as containing smooth muscle in both the longitudinal and circular systems. Addition of barium chloride to the bath had no effect on the esophagus.

Administration of pituitary liquid (Armour's) in dilution of 1:100,000 is followed by inhibition of both circular and longitudinal systems

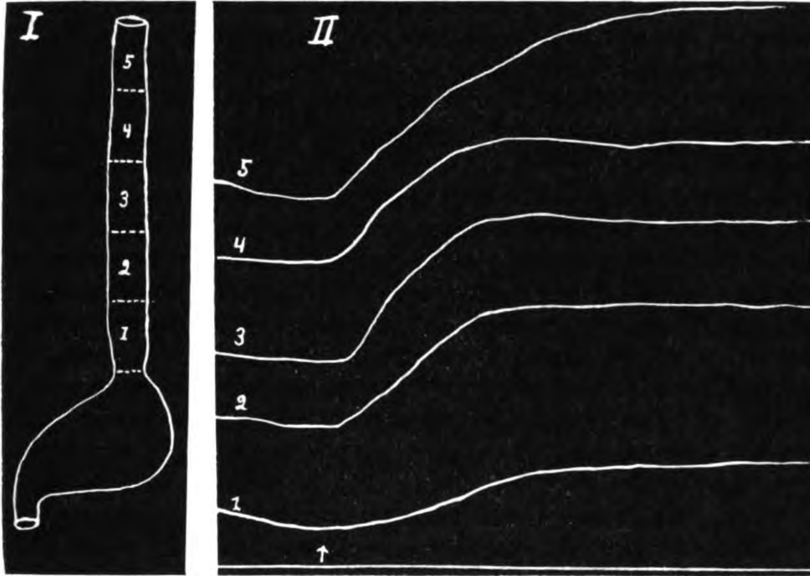


Fig. 6. Turtle esophagus. *I*, diagram of esophagus and stomach indicating relationships of rings used in this experiment. *II*, stimultaneous record of isolated rings of circular neuro-muscular system as indicated. Arrow indicates point of application of adrenalin (1:100,000) showing apparent decreased response of this system to adrenalin as region of cardia is approached.

(fig. 2, *D*). This confirms the work of Waddell (9) but it is not certain whether this effect is the result of the extract of the pituitary gland or of the histamine which this preparation is said to contain. This might account for the failure of this preparation as a specific myotropic drug.

Results on the turtle's esophagus: *1. Adrenalin.* The action of adrenalin on the esophagus of the turtle is essentially the same for both longitudinal and circular systems. The addition of adrenalin in a dilution of 1:100,000 to preparations of turtle's esophagus is followed by a marked rise in tone (fig. 5, *Ad*). In many cases there is a distinct

Digitized by Google

initial inhibition of the tone preceding the stimulation. This effect does not appear in preparations with poor tone. The stimulating effect of adrenalin is more marked on the circular system than on the longitudinal system, and in both it continues for a long period of time in spite of repeated washings with adrenalin-free Ringer's solution. These reactions occur in preparations taken from all levels of the esophagus. In many preparations of the circular system there appears to be decrease in the response to adrenalin in those rings taken from the region of the cardia as compared with those taken from higher levels (fig. 6). All adrenalin reactions are obtained after atropine paralysis of the vagi.

2. Pilocarpine. The influence of pilocarpine in dilutions of 1:100,000 is the same for both circular and longitudinal systems. The drug causes a temporary inhibition of the tone, the degree of the inhibition depending on the amount of tone maintained by the preparation before the drug was added. If the circular system is in a condition of maximum contraction as the result of the administration of adrenalin, inhibition is the only effect produced by the administration of pilocarpine (fig. 5, *P*). In preparations with moderate tonus the initial inhibition is followed by a rise in tone (Fig. 5, *Lp*) and occasionally rhythmic contractions.

These pilocarpine reactions do not occur after atropine and are promptly antagonized by that drug (fig. 5, *At.*).

These results confirm the observations of Carlson and Luckhardt (1) who noted that stimulation of the vagi in turtles was followed by an inhibition of the tonus at least of the upper two-thirds of the esophagus. They also observed esophageal inhibition after pilocarpine injections. In their work they did not observe a motor effect from vagus stimulation or pilocarpine injections except in one case in which the animal was moribund and the esophagus in poor tone. This series appears to confirm the accuracy of their observation of that one case.

3. Nicotine. The addition of nicotine in dilution of 1:200,000 to the preparations of turtle's esophagus is followed by a profound inhibition of tone (fig. 5, *N*). This is more marked in the circular system and is dependent on the degree of tone maintained by the preparation. In many cases the inhibition is followed by an increase in tone.

4. Histamine. Histamine causes stimulation of the preparations of turtle's esophagus.

Discussion of results: *1. Dependence of the frog's neuro-muscular system on oxygen.* The neuro-muscular system of the frog's esophagus

Digitized by Google

is very sensitive to changes in its oxygen supply. This is especially true of the circular system when it is in its normal position inside of the longitudinal system. Turning the preparation inside out reduces this marked sensitivity. It gives the circular system more ready access to the oxygen in the bath. That this is an important factor is shown by the fact that it was necessary as a routine measure to turn all preparations inside out before any dependable reactions could be secured with the drugs. Preparations of the frog's esophagus will not develop spontaneous activity when atmospheric air is used instead of pure oxygen thereby indicating the dependence of this system on oxygen.

This is in marked contrast to the resistance to anemia of the nerve cells in the mesenteric plexus in cats, as pointed out by Cannon and Burket (4). These investigators showed that complete anemia of parts of the alimentary canal produced by compression between glass plates may last as long as three hours without loss of normal activity or of nerve cells from the compressed area.

The dependence on oxygen of the neuro-muscular system of the frog's esophagus is striking in its similarity to the dependence on oxygen supply of the centers for spinal reflexes as pointed out by Sherrington (5).

The gradual development of contractions in the isolated preparations indicates a period of recovery from the shock to the isolated peripheral nervous mechanism of making the isolated preparation. A similar condition was pointed out by Alvarez (6) who showed that strips taken from the cardiac regions of the frog's stomach suffer most from the trauma of excision and recover slower as compared to strips from the antrum. In the turtle this shock seems to be more profound. This apparent shock effect is also strikingly similar to that experienced in the spinal reflex centers.

In a previous report (7) attention was called to the fact that repeated stimulation of the vagi in turtles was followed by a rapid failure of gastric contractions in response to that stimulation. This seemed to indicate either a rapid fatigue or the development of some sort of a refractory condition within the vago-gastric motor mechanism. Both of these effects are similar to the corresponding points in Sherrington's analysis of spinal reflexes in mammals. In a later report (8) attention was called to the complexity of the peripheral neuro-muscular system in the turtle's stomach.

Digitized by Google

It would seem, therefore, that in these preparations we are dealing with an isolated nervous center which is sensitive to oxygen want and also to shock effects and that this center is controlling the spontaneous activities of the isolated neuro-muscular system of the frog's esophagus.

2. Nature of the preliminary inhibition. It was noted above in the case of nicotine on the frog's esophagus and at times on the turtle's esophagus that there was a primary inhibition which preceded the stimulating effects. This also occurred in the case of adrenalin and pilocarpine in the turtle.

The drug actions are all essentially the same on both systems. Both systems are present and potent. The delicate lever records primarily one neuro-muscular system but not to the complete exclusion of the other. It would seem therefore that the effect recorded in any case would be the algebraic sum of the activities of both neuro-muscular systems. This might be responsible for at least a portion of the primary inhibition recorded.

On the other hand it appears that we are dealing with a complex isolated nervous center capable of both inhibitory and motor activities. Assuming, at least for the present, that this is true and that the center is in a condition of tonic activity *the primary reaction of either system to the drug will depend at least in part on the relative tonus of the two systems.*

The circular and the longitudinal systems are, at least in part, mutually antagonistic, so that according to the law of reciprocal innervation, tonus or activity of the one should lead to depression of the other. Furthermore, the primary response of either system to drugs may be reversed by a reversal of the tonic state at the time of application of the drug.

Finally, it may be a case of double innervation of the musculature, the inhibitory innervation having a shorter latent period than the motor innervation. In that event both reactions would occur in a definite time relationship to each other which is never reversed.

3. Apparent failure of the so-called specific myotropic drugs to stimulate the smooth muscle of the frog's esophagus. It is noticed in the case of histamine, pituitary liquid and barium chloride that the usual stimulating effects were not obtained. There is also a striking similarity in the action of histamine on the frog's esophagus to that of adrenalin. A glance at table 1 will reveal a difference in the action of histamine on the frog's esophagus and on the turtle's esophagus. In the latter

Digitized by Google

animal the action is similar to that which is usually expected from histamine. As pointed out by Carlson and Luckhardt (1) "the conditions found in one animal group or species do not necessarily apply

TABLE 1

The action of drugs on preparations of the esophagus. Stimulation = +; inhibition = −; when both inhibition and stimulation were obtained in the same preparation, the primary action is placed above

	FROG ESOPHAGUS		TURTLE ESOPHAGUS	
	Circular system	Longitudinal system	Circular system	Longitudinal system
Non-aerated Ringer's solution	−			
Aerated Ringer's solution	−			
Oxygenated Ringer's solution	+*			
Adrenalin	+ (14 cases) − (7 cases)	−	− ++ all levels	− ++
Pilocarpine	+	+	− ++	++ −(after adrenalin)
Nicotine	− ++	− ++		
Atropine	−(after pilocarpine)	−(after pilocarpine) 0 −(temporary)		
Histamine	−	−	+	+
Pituitrin	−	−		

* The + in this case means that spontaneous activity developed.

to another group or species as the degree of differentiation in the motor control from the primitive condition appears to vary greatly in different animals."

Digitized by Google

4. Results on the intact animal as compared with the isolated preparations. It is evident that if the activities of the esophagus with its extrinsic innervation sectioned is dependent on a peripheral center the preparation will be more physiological if left in the animal with its circulation intact and without the traumatism necessary to isolate a strip or ring. In spite of the abnormal conditions surrounding the isolated preparation there is a striking confirmation of the results of Carlson and Luckhardt (1) on the physiological animal. The main difference seems to be in the magnitude of the reactions rather than their character and this may be easily explained on the basis of the difference in the recording systems.

SUMMARY AND CONCLUSIONS

Frog's esophagus. 1. Tonus and spontaneous contractions are maintained only in well oxygenated Ringer's solution. Atmospheric air cannot be substituted for pure oxygen. The circular system is more sensitive to lack of oxygen than to longitudinal system when in its normal position. Turning the esophagus inside out permits better aeration and more ready access of drugs to the circular system.

2. Adrenalin causes a uniform inhibition of the longitudinal system and stimulation (66 per cent of experiments) or inhibition (33 per cent of experiments) of the circular system. The adrenalin action seems to be more marked after atropine.

3. Pilocarpine stimulates both circular and longitudinal systems. Atropin counteracts this effect of pilocarpine.

4. Nicotine causes a primary inhibition followed by stimulation of the circular and longitudinal systems.

5. Histamine and pituitary liquid (Armour's) cause uniform inhibition of the circular and the longitudinal systems.

Turtle's esophagus. 1. The longitudinal and circular systems of the turtle's esophagus at all levels react the same to the drugs investigated.

2. Administration of adrenalin causes a profound and lasting stimulation usually preceded by a temporary inhibition. Atropine has no effect on this adrenalin action.

3. Pilocarpine causes a temporary inhibition followed by a marked stimulation. Only inhibitory effects occur in the circular system in preparations in a condition of maximum contraction following the administration of adrenalin. The preliminary inhibition may be absent if the tone is low.

Digitized by Google

4. Nicotine causes a profound inhibition. Following this in some cases there is an increased tone and then gradual recovery to the initial level.
5. Histamine is followed by an increase in tone.

The author wishes to take this opportunity to express his deep appreciation to Dr. A. J. Carlson and Dr. A. B. Luckhardt for their inspiration, for invaluable suggestions and unstinting help throughout the entire progress of this and previous studies in this series.

BIBLIOGRAPHY

(1) Carlson and Luckhardt: This Journal, 1921, lvii, 299.
(2) Luckhardt and Carlson: This Journal, 1920, lv, 122.
(3) Ecker and Wiedersheim: Anat. des Frosches, ii, Brauns., 1904, 72.
(4) Cannon and Burket: This Journal, 1913, xxxii, 347.
(5) Sherrington: Integrative action of the nervous system, New Haven, 1906.
(6) Alvarez: This Journal, 1916–17, xlii, 422.
(7) Bercovitz and Rogers: This Journal, 1921, lv, 323.
(8) Rogers and Bercovitz: This Journal, 1921, lvi, 257.
(9) Waddell: This Journal, 1916, xli, 529.

Digitized by Google

THE WATER CONTENT OF THE TISSUES IN EXPERIMENTAL BERIBERI

DOROTHY JOSEPHINE KRAUSE

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 28, 1921

The object of this investigation was to ascertain whether there is an increase in the water content of the tissues in experimental beriberi, because of its bearing on edema as a phenomenon in starvation, protein deficiency (1) and other forms of maladies due to faulty diets. Our working hypothesis, as outlined by Doctor Carlson, was to the effect that incipient edema may be a consequence of all dietary deficiencies (quantitative and qualitative), macroscopically recognized edema appearing as a final symptom in a certain percentage of the individuals; but if the water content of the tissues is determined quantitatively, incipient edema may be universal. The work was started on beriberi, because the literature recognizes two types of this dietary disease, at least in man, the wet beriberi (edema) of infants, and the dry beriberi or polyneuritis of adults.

LITERATURE. Hirsch's Handbook in 1885 (2) mentions edema in legs, serous cavities and in lungs in some clinical cases of beriberi. Holst (3) occasionally found moderate edema under skin of legs and feet of polyneuritic pigeons. Holst and Frölich (4) noted frequent subcutaneous edema in scurvy guinea-pigs also. They call attention to a resemblance between abortive scurvy of guinea-pigs and ship beriberi (of man). Vedder and Clark (5) found little evidence of edema in polyneuritic chickens, except in cases of severe prostration, and then only a slight edema in pericardium at the base of the heart. Matsouka (6) finds that a congestion edema of the lungs is a clinical characteristic of beriberi. This goes hand in hand with a beginning dilatation and hypertrophy of the right heart. He also mentions presence of hydrothorax. Gibson and Concepcion (7) consider that hypertrophy of the heart and edema are typical of infantile beriberi, and mention signs of edema in beriberi dogs and pigs. Darling (8) observed edema of lungs in scurvy in Africa. Hess (9) spoke of subcutaneous edema in infantile scurvy, a widespread edema that does not pit on pressure, as it

Digitized by Google

infiltrates layers of integument and muscle. He noted a close relationship between this and infantile beriberi. McCarrison (10) in a study of the effect of deficiency diet on organs, finds in some of the pigeons such symptoms as these: excess fluid in the pericardial sac, band of edema at auriculo-ventricular juncture, edema in the lungs, some congestion in the lower pole of the left kidney, and edema in the groin. Effusions into serous sacs, although found frequently at autopsy, were rarely recognized clinically. He finds these symptoms in pigeons, not only after a diet of polished rice, but after a period of starvation. He also observes acidosis when antineuritic vitamine is absent. After diet of polished rice McCarrison (11) reports that the intestines of pigeons showed atrophy and congestion especially in the upper part, and occasionally he could see an edematous infiltration of the coats of the bowel. In scurvy guinea-pigs there was chiefly a hemorrhagic infiltration. He notes a relation between the form of beriberi occurring, and condition of suprarenals (12). Kellaway (13) confirmed McCarrison's findings. Bigland (14) in relation to edema, emphasizes effect of a food deficiency on certain endocrine organs. Rommel and Vedder (15) mention serous fluid in the pericardial sac and in the thoracic and abdominal cavities, and edematous lungs, occurring in wet beriberi in the pig. Williams and Saleeby (16) mention edema as a clinical symptom appearing frequently in beriberi, especially in infants. All these references to the presence of edema in beriberi and scurvy are based on macroscopic examination, except in a few cases.

Experimental methods. Beriberi was induced in the chickens and pigeons by feeding polished rice exclusively. Sand and water were also provided. The cages were large, and two to four birds were kept in a cage. Temperatures were taken before and after appearance of disease, and the typical fall in temperature occurred in every case. Just before the last stage of the disease, the animals were killed by decapitation and allowed to bleed dry. They were then opened up, and the organs dissected out and put in crucibles (previously dried and weighed). The crucibles and organs were weighed, heated in an oven (120°C.) till the weight became constant and the percentage of moisture computed. The same procedure was followed with controls. Animals used were over one year old, so the age factor was eliminated. The diet of the controls was unpolished rice. The organs studied were the brain, lungs, heart, liver, spleen, testes, kidneys, pancreas, stomach, intestines, muscle and skin. The brain was removed from its meninges, and cut off below medulla and excluding hypophysis. The lungs were

Digitized by Google

removed from the pleural sac. The heart was taken out of the pericardial sac, opened up and blood clots removed. Stomach and intestines were slit open, contents washed out, and adhering moisture removed by placing between two sheets of drying paper (in some series all of the intestine was used; and in others segments only). Muscles were taken from back of leg. Skin covering the leg was used. With chicks the gall bladder was dissected from the liver. In all cases masses of fat clinging to organs were removed, as the water content varies somewhat with lipoids. (17).

In the rats beriberi was induced by feeding a synthetic diet lacking in water-soluble B. The diet used was based upon that of McCollum and Davis (18), but with increased percentage of protein and butter fat to cover those factors. It was:

	per cent
Butter fat	12.0
Casein	18.0
Polished rice	66.5
Salt mixture	3.5
Agar-agar	about 1 per cent added for bulk

Salt mixture used was the one found satisfactory by McCollum and Davis, namely,

	grams
$NaCl$	5.0
K_2HPO_4	12.10
$CaH_4(PO_4)_2$	2.5
Ca lactate	29.5
Fe citrate	1.0

The rice (with agar-agar) was boiled in tap water till kernels were fairly soft, then salt mixture, casein, and butter fat added. The rats were given this mixture ad libitum, in addition to fresh water. Controls were given a normal diet of milk, oats, bread, carrots and greens.

Guinea-pigs were used for both beriberi and scurvy experiments. Beriberi was induced on the synthetic diet fed to the rats, but as guinea-pigs are reported (in the literature) to develop scurvy on a diet which causes beriberi in birds, they were given daily 5 cc. of orange juice extracted with Fuller's earth according to method of Hardin and Zilva (19). This is supposed to remove water-soluble B and leave the antiscorbutic factor. To induce scurvy in the guinea-pigs, bran and hay were fed ad libitum. In all the guinea-pigs, except four, the same method of killing and determining water content of organs

Digitized by Google

was used as in case of the chickens and pigeons. Four of the guinea-pigs and all of the rats were dried whole, after being killed with ether. In the rats the sciatic nerves were removed, and stained for degeneration by the Marchi method.

RESULTS. *Chickens.* Two sets of chickens were used: six chickens in the first set, and five in the second. (One chicken in each set did not develop beriberi during the duration of the experiment.) The first set was started on October 21, 1920. Losses in weight of the beribery chickens were as follows: 1175 grams to 1055 grams; 1365 grams to 1035 grams; and 1635 grams to 1110 grams. The birds gained weight for the first week or two of the feeding. Beriberi chickens were killed on November 29, 19 and 24, respectively. Two were in well-advanced stages, and a third in the last stage of the disease. The second set was started on December 24, 1920. Losses in weight were: 1935 to 1230 grams; and 1935 grams to 1570 grams. These also gained in weight for the first week or ten days. These beriberi chickens were killed on January 25 and 26, respectively. In no case was edema noted at post mortem.

The average water contents of the beriberi chickens and controls are given in table 1.

These results show a tendency toward increased water content in the skin, but the results are not constant enough to warrant a definite conclusion. No increase is seen in the other organs. In fact in some cases the total loss in water content more than balances the total gain.

Pigeons. Beriberi was induced in two sets of pigeons; six in the first and five in the second. The first set was started on October 21, 1920. Losses in weight of the beriberi pigeons were: 347 grams to 245 grams; 285 grams to 140 grams; 275 grams to 181 grams; and 360 grams to 198 grams. They gained in weight for the first week or ten days. The beriberi birds were killed on November 10, 22, 23 and 27, respectively. The second set was started November 19, 1920. Losses of weight of beriberi pigeons in this set were 320 grams to 210 grams; 315 grams to 250 grams; and 275 grams to 188 grams. They gained in weight for two to three weeks. These beriberi birds were killed on December 8 and January 3 and all were in fairly advanced stages. No signs of edema was observed at autopsy. The average water contents of the organs are given in table 2.

A study of table 2 shows in beriberi pigeons a distinct increase of water content in certain organs, and a somewhat slight but definite tendency toward it in other organs. The heart and intestines show the greatest

Digitized by Google

and most invariable increase. A rather constant tendency toward increased water content is seen in the lungs, skin, muscles and kidneys. The testes show a significant decrease in water content.

TABLE 1

Water content

		Control chickens			Beriberi chickens			H_2O + or −
		High	Low	Average	High	Low	Average	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	Brain	79.50	79.00	79.25	79.58	77.98	78.90	− 0.68
	Lung	80.50	80.30	80.40	75.75	72.10	73.95	− 1.45
	Heart	74.50	70.60	72.55	72.30	64.58	69.36	− 3.19
	Muscle	78.90	75.50	77.20	76.50	72.50	75.13	− 2.07
	Skin	56.01	50.30	53.15	60.20	55.10	57.68	+ 4.53
	Stomach	74.00	72.40	73.20	71.50	68.30	69.63	− 3.57
	Spleen	84.12	76.73	80.43	75.49	74.90	75.19	− 5.24
	Pancreas	71.80	67.50	69.65	71.28	69.08	70.32	+ 0.67
	Intestines	79.90	77.00	78.45	77.50	75.17	76.50	− 1.95
	Kidney	76.65	76.55	76.60	77.80	74.70	76.66	+ 0.06
	Testes			83.40	79.60	75.0	77.37	− 6.03
	Liver	75.25	71.60	73.43	72.20	69.28	70.59	− 2.84
	Adrenals	76.60	69.77	73.19			59.00	−14.19
II	Brain	79.50	61.82	70.66	81.00	80.30	80.70	+10.04*
	Lung	78.10	76.40	77.30	77.10	77.01	77.05	− 0.25
	Heart	76.85	75.80	76.33	83.05	73.0	78.0	+ 1.67
	Muscle	76.60	75.50	76.05	75.60	74.20	74.90	− 1.15
	Skin	59.90	58.75	59.33	68.95	68.60	68.78	+ 9.45
	Stomach	75.70	73.50	74.60	74.45	72.64	73.55	− 1.05
	Spleen	77.75	77.25	77.50	78.20	76.55	77.45	− 0.05
	Pancreas	71.90	71.50	71.70	69.50	50.90	60.20	−11.50
	Intestines	77.25	75.95	76.60	78.90	78.40	78.65	+ 2.05
	Kidney	79.75	77.49	78.62	81.48	79.85	80.66	+ 2.04
	Testes	85.50	84.50	85.0	80.0	79.0	79.50	− 5.50
	Liver	74.10	69.90	72.0	74.75	71.25	72.45	+ 0.45
	Adrenals			67.0			73.0	+ 6.00

* Positive values not significant as one result far below all other values of brain.

Series I: 2 control chickens; 3 beriberi chickens.

Series II: 2 control chickens; 2 beriberi chickens.

Another set of pigeons was *starved* till loss in weight was equal to the average loss in the beriberi birds, to see if partial starvation rather than

Digitized by Google

the beriberi condition is the causal factor. The results are given in table 3.

It is evident from table 3 that starvation leads to an increased water content in the organs comparable to that in beriberi. This may be due to the acidosis of inanition.

TABLE 2

Water content

		CONTROL PIGEONS			BERIBERI PIGEONS			H_2O + OR −
		High	Low	Average	High	Low	Average	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	Brain	80.07	74.00	77.03	83.30	79.80	81.02	+ 3.99
	Lung	76.99	76.70	76.84	83.45	79.28	82.92	+ 6.08
	Heart	72.30	71.75	72.03	81.30	78.05	79.23	+ 7.20
	Muscle	75.15	72.30	73.73	77.30	74.40	75.80	+ 2.07
	Skin	41.17	31.10	38.64	69.85	64.55	66.10	+27.46
	Stomach	70.15	67.55	68.85	72.10	70.80	71.30	+ 2.45
	Spleen	75.75	75.48	75.61	84.60	62.50	72.71	− 2.90
	Pancreas	70.85	69.70	70.28	71.60	69.25	70.31	+ 0.03
	Intestines	74.58	65.50	70.04	84.40	81.25	82.48	+ 8.44
	Kidney	74.20	73.15	73.68	80.15	77.30	78.63	+ 4.95
	Testes			76.50			61.58	−15.02
	Liver	71.48	71.40	71.44	76.18	72.0	74.17	+ 2.73
II	Brain	80.80	80.0	80.40	80.25	79.44	79.86	− 0.54
	Lung	77.25	77.15	77.20	82.0	78.40	79.70	+ 2.50
	Heart	74.95	74.55	74.75	81.49	78.80	80.56	+ 5.81
	Muscle	73.60	72.60	73.10	76.90	75.0	76.18	+ 3.08
	Skin	45.42	14.77	30.10	65.75	61.55	62.97	+32.87
	Stomach	70.65	69.38	70.02	72.10	71.05	71.57	+ 1.55
	Spleen	78.60	70.95	74.78	71.68	67.90	70.06	− 4.72
	Pancreas	71.60	71.60	71.60	73.28	71.45	72.09	+ 0.49
	Intestines	72.60	68.15	70.33	81.55	78.00	79.98	+ 9.65
	Kidney	77.45	75.20	76.33	77.90	78.12	77.97	+ 1.64
	Testes	85.10	71.65	78.38	69.40	60.0	64.70	−13.68
	Liver	76.75	72.58	74.67	75.10	72.25	74.01	− 0.66

Series I: 2 control pigeons; 4 beriberi pigeons.
Series II: 2 control pigeons; 3 beriberi pigeons.

Guinea-pigs. Only two guinea-pigs were used in our attempt to produce beriberi, as it is not certain that the disease can be experimentally induced in guinea-pigs. We found no reference in the litera-

Digitized by Google

ture to beriberi in this species. One guinea-pig was used as a control. One of the experimental animals was lost by not being familiar with the symptoms of beriberi in the guinea-pig. The diagnosis of beriberi was checked by staining the sciatic nerves for degeneration (Marchi method). The symptoms observed in the guinea-pigs on the above diet were as follows: Gain in weight (equal to that of control) for three to four weeks; and then quick loss in weight and death in four to five days. Both guinea-pigs showed roughness of hair after four weeks

TABLE 3

Water content

	TWO CONTROL PIGEONS			FOUR STARVED PIGEONS			H_2O + OR −
	High	Low	Average	High	Low	Average	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	79.85	79.49	79.67	81.00	79.80	80.54	+ 0.87
Lung	77.45	76.75	77.22	82.90	74.45	79.32	+ 2.10
Heart	75.05	74.10	74.60	79.80	77.55	78.80	+ 4.20
Muscle	75.65	74.49	75.07	75.30	75.40	77.50	+ 2.43
Skin	62.90	61.60	62.25	70.70	60.50	65.93	+ 3.68
Stomach	71.75	69.10	70.43	70.75	68.05	69.49	− 0.94
Spleen	81.35	73.35	77.35	66.70	57.50	61.80	−15.55
Pancreas	72.60	71.73	72.17	75.50	71.51	73.44	+ 1.27
Intestines	70.95	70.70	70.83	78.30	74.95	76.44	+ 5.61
Kidney	77.01	74.70	75.86	81.10	76.55	78.80	+ 2.96
Testes			85.98	79.50	68.45	73.98	−12.0
Liver	68.80	68.50	68.65	74.48	71.52	72.94	+ 4.29*

* Positive values not significant when water content of the controls is compared with controls of table 2.

on the diet. From this on the animals remained in about the same condition and maintained a good appetite, till appearance of definite symptoms of the disease. But the animals became gradually more noisy when they were being fed. In the guinea-pig used, definite symptoms appeared at the end of the sixth week. The animal became very weak, and was uncertain in his movements (stupid) with subnormal temperature, and absence of the usual nervous shivering on being handled. No signs of scurvy were seen on autopsy, showing that the extracted orange juice had protected him from scurvy. Results are given in table 4.

Table 4 reveals no increased water content in any organ.

Digitized by Google

TABLE 4

Water content

	ONE CONTROL GUINEA-PIG	ONE BERIBERI GUINEA-PIG	H_2O + OR −
	per cent	*per cent*	*per cent*
Brain	80.90	80.60	−0.30
Lung	80.60	81.10	+0.50
Heart	79.35	79.43	+0.08
Muscle	77.75	76.10	−1.15
Skin	70.50	63.05	−7.45
Stomach	79.0	80.80	+1.80
Spleen	79.10	78.60	−0.50
Intestine	87.30	85.30	−2.0
Kidney	78.85	79.40	+0.55
Liver	74.35	76.60	+2.25

Scurvy was developed in two series of guinea-pigs. In the first series six animals were used. They were killed after being on the special diet for two to three weeks. The animals lost weight, first slowly, then more rapidly. Other external manifestations were roughness of hair, sitting crouched up, and in some cases, squealing when handled. Diagnosis of scurvy was confirmed by typical appearance on autopsy. The results on this first series are given in table 5.

TABLE 5

Water content

	THREE CONTROL GUINEA-PIGS			THREE SCURVY GUINEA-PIGS			H_2O + OR −
	High	Low	Average	High	Low	Average	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	81.10	71.98	80.46	80.90	80.15	80.75	+0.29
Lung	81.49	51.60*	71.15	80.60	79.90	80.30	+9.15
Heart	81.30	80.10	80.00	80.70	79.20	80.20	−0.60
Muscie	77.95	77.20	77.53	76.95	76.06	76.51	−1.02
Skin	69.95	67.50	68.72	71.60	64.03	67.57	−1.15
Stomach	81.40	80.10	80.90	82.0	80.50	81.50	+0.60
Spleen	79.75	78.08	79.74	79.45	74.05	77.27	−1.47
Intestines	85.70	82.90	84.50	65.60	84.45	84.88	+0.38
Kidney	84.55	77.80	80.58	81.55	80.75	81.08	+0.50
Liver	76.60	74.60	75.73	74.90	70.75	73.24	−2.49
Adrenals	64.45	57.00	61.03	75.50	60.90	67.20	+6.17

* This result probably not correct as it is so much lower than the rest.

Digitized by Google

Table 5 reveals no definite edema in any organ in the scurvy guinea-pigs.

In our method of studying the separate organs, the serous sacs and cavities are not included in the determinations, hence there might be some edema that our method fails to disclose. In order to test this

TABLE 6

Water content of entire body

TWO CONTROL GUINEA-PIGS	TWO SCURVY GUINEA-PIGS	INCREASE IN H_2O
per cent	*per cent*	
74.17	77.60	+3.43
73.63	76.70	+3.07

possibility, scurvy was produced in two additional guinea-pigs, and two animals used as controls. The animals were killed by ether and desiccated in toto (including the blood). The results are given in table 6.

Rats on beriberi diet. Eight rats were employed in this test, two serving as controls. They weighed between 50 to 80 grams at the beginning of the experiment, and were nearly of the same age, but as all were over five weeks old, age would be eliminated as a factor (20). After nine weeks on the deficient diet, they were killed with ether and dried

TABLE 7

Water content of entire body

TWO CONTROL RATS	SIX BERIBERI RATS	H_2O + OR −
per cent	*per cent*	*per cent*
70.07	69.50	−0.29
69.50	69.25	−0.54
	71.25	+1.46
	72.53	+2.74
	72.40	+2.61
	73.0	+3.21

whole as described above. The rats did not gain as well as the controls, but otherwise showed no external symptoms. There was some degeneration of the sciatic nerve in every animal, showing the lack of water-soluble B had produced the nervous lesions of polyneuritis. The results are shown in table 7.

In four of the rats there is a small but definite increase in water content. The remaining two are practically identical with the controls.

Digitized by Google

CONCLUSIONS

1. There is no definite increase in water content of the organs of beriberi chickens, except possibly in the skin.

2. In beriberi pigeons there is an increased water content in the intestines, and in the heart, the lungs, the skin, the muscles and the kidneys. Starvation, in proportion to the loss in weight in the beriberi birds, leads to a similar increase of water in these tissues.

3. In one beriberi guinea-pig, no increased water content was found in the organs studied.

4. In scurvy guinea-pigs, increased water content was not detected in determinations of separate organs, but when the animals were dried whole, such a tendency was noted.

5. In beriberi rats (entire body), there was increased water content in some cases and in others there was no change.

6. Quantitative analysis (separate organs, or the entire animal) appears to reveal increased water content of tissues (or incipient edema) in dietary deficiency diseases, where edema is not in evidence on the usual gross examination.

BIBLIOGRAPHY

(1) Kohman: This Journal, 1920, li, 378.
(2) Hirsch: Handbook of geographical and historical pathology, 1885, ii.
(3) Holst: Journ. Hyg., 1907, vii, 618.
(4) Holst and Froelich: Journ. Hyg., 1907, vii, 618.
(5) Vedder and Clark: Phil. Journ. Sci., 1912, vii, 423.
(6) Matsouka: Journ. Path., 1915–16, xx, 191.
(7) Gibson and Concepcion: Phil. Journ. Sci., 1916, xi, 119.
(8) Darling: Journ. Amer. Med. Assoc., 1914, lxiii, 1290.
(9) Hess: Journ. Amer. Med. Assoc., 1915, lxv, 1003.
(10) McCarrison: Ind. Journ. Med. Res., 1919, vi, 275.
(11) McCarrison: Ind. Journ. Med. Res., 1919, vi, 167.
(12) McCarrison: Brit. Med. Journ., 1920, x, 236.
(13) Kellaway: Proc. Roy. Soc., 1921, xcii, B, 6.
(14) Bigland: Lancet, 1920, i, 243.
(15) Rommel: Journ. Agric. Res., 1915–16, v, 489.
(16) Williams and Saleeby: Phil. Journ. Sci., 1915, x, 99.
(17) Mayer and Schaeffer: Journ. de Physiol., 1914, xvi, 23.
(18) McCollum and Davis: Journ. Biol. Chem., 1915, xxiii, 181.
(19) Harden and Zilva: Biochem. Journ., xii, 193.
(20) Donaldson: Journ. Comp. Neurol., 1910, xx, 119.

Digitized by Google

STUDIES IN CARBON MONOXIDE ASPHYXIA

II. The Growth of Neuroblast in the Presence of Carbon Monoxide

A DEMONSTRATION THAT THIS GAS HAS NO DIRECT TOXIC ACTION UPON NERVOUS TISSUE

HOWARD W. HAGGARD

Investigations performed for the U. S. Bureau of Mines in the Laboratory of Applied Physiology, Yale University, New Haven

Received for publication December 6, 1921

In severe carbon monoxide asphyxia the chief damage is usually wrought upon the central nervous system. This apparently selective action of the gas led to the belief that carbon monoxide exerts a specific toxic action upon nervous tissue. From the clinical and pathological sides the acceptance of this specificity affords an easy explanation of an otherwise difficult problem. Physiological evidence indicates, however, that aside from its displacement of oxygen from the blood, carbon monoxide is as innocuous as nitrogen (1). The nervous sequelae of the poisoning follow as one of a train of profound physiological alterations, due primarily to the anoxemia induced by the combination of carbon monoxide with hemoglobin and exclusion of oxygen.

In the present work an attempt has been made to obtain some direct evidence upon this problem by observing the action of carbon monoxide upon cultures of chick neuroblasts growing in vitro. The nerve cells under these conditions maintain their gaseous exchange directly through the plasma, in which they are suspended, without the intervention of hemoglobin. By thus dispensing with hemoglobin, the asphyxiant action of the carbon monoxide is avoided. The concentration of the gas in the medium about the cells is determined solely by the tension of gas in the atmosphere with which the plasma is in contact. For this reason it is possible to expose the neuroblasts to far greater amounts of carbon monoxide than would be possible in the body.

As will be seen in the following experimental work carbon monoxide even in concentrations as high as 79 per cent acts as an inert gas toward

Digitized by Google

nerve cells. When the carbon monoxide is supplied in the form of illuminating gas, i.e., fattened water gas, this is not the case. Some accessory substance in this gas has, at least under in vitro conditions, a highly toxic action upon growing nerve cells.

Experimental methods. The general technique of preparation and the normal appearance of cultures of chick nervous tissue grown in vitro have been fully described by R. G. Harrison (2) and his pupils (3). I take this opportunity to express my indebtedness to Professor Harrison for his personal advice and suggestions during the course of this work. In brief the technique is as follows: Fertile hen's eggs are incubated at 39°C. for 48 hours or longer. One of these eggs is then placed upon its side, and the upper surface sterilized by washing with alcohol and dried.

TABLE 1

Showing the reaction of chick nervous tissue in the presence of air, carbon monoxide, and air with 0.1 per cent illuminating gas

ATMOSPHERE TO WHICH NERVOUS TISSUE WAS EXPOSED	NUMBER OF CULTURES	NUMBER OF GROWTHS	PER CENT GROWTH	REMARKS
Atmospheric air	36	31	86.1	Growth normal in appearance
79 per cent carbon monoxide and 21 per cent oxygen	20	18	90.1	Growth normal in appearance
Atmospheric air and 0.1 per cent illuminating gas	18	0	0	Tissue dead and partially degenerated

An area of the shell about 3 cm. in diameter is cut away with scissors, thus exposing the chick embryo. Under strong illumination and a binocular microscope small segments of neuroblastic tissue are cut away with sharp-pointed iridectomy scissors. These fragments are floated into warm saline and cut into smaller bits. One of these is then drawn into a capillary pipette and placed in a drop of chicken plasma resting upon a microscope cover slip. Asepsis is maintained throughout. The plasma employed as the supporting medium for the tissue is obtained by centrifuging cooled chicken blood; the blood is drawn by means of a Luer syringe and needle from the superficial vein upon the medial aspect of a fowl's wing.

The cover slip with its drop of plasma containing the tissue is inverted over a glass ring the surfaces of which are coated with petrolatum.

Digitized by Google

This in turn rests upon a microscope slide. Thus sealed, the hanging drop culture is aerated only by the atmosphere within the cell formed by the ring, the slide, and the cover glass. But in the present work the slide used was pierced with a 2 mm. central hole opening into this cell.

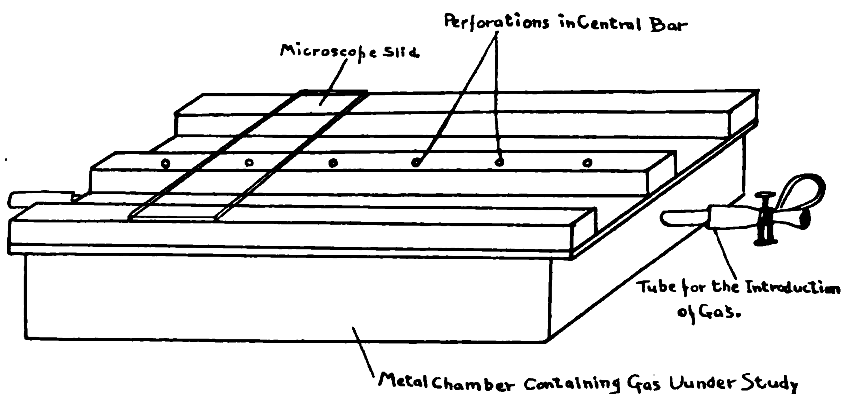


Fig. 1. Apparatus for exposing in vitro cultures to gases

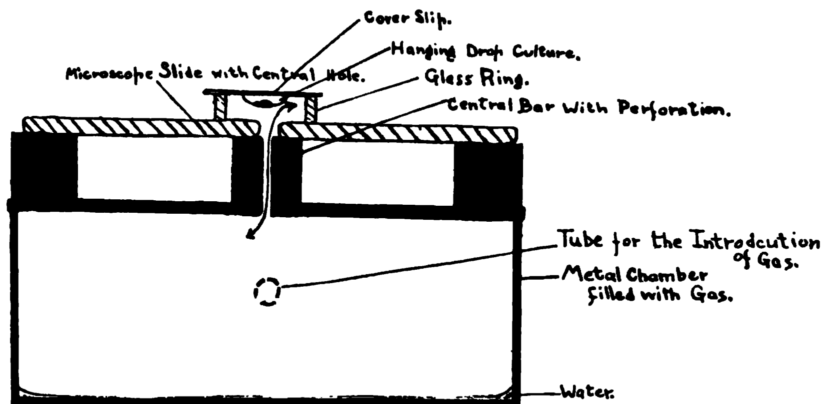


Fig. 2. Sectional view of apparatus showing culture in place

In order to study the effects of gases other than atmospheric air upon the growing nervous tissue it is necessary to employ some means of introducing and maintaining the gas within the cell. For this purpose the following apparatus was designed: It consists (figs. 1 and 2) of a metal chamber acting as a gas reservoir, which is surmounted by three metal bars placed equidistant and extending longitudinally. The

Digitized by Google

middle one of these bars is perforated at 3 cm. intervals with small openings which lead to the chamber below. The chamber is made of sheet brass and is 25 cm. long, 10 cm. wide and 4 cm. deep. The bars are of square brass, 1.5 cm. thick and extend the full length of the chamber. At either end of the metal chamber are small tubes fitted with short lengths of rubber tubing and pinch clamps.

The chamber is sealed by coating the bars lightly with petrolatum and placing microscope slides transversely across the three in such a manner as to cover the openings. The gas under study is introduced by connecting one of the tubes to a gasometer filled with the desired mixture. With both pinch clamps open, a sufficient volume is passed through to afford thorough flushing. The effluent tube is then connected to a gas analyzer and a sample drawn for analysis, after which both clamps are closed. The atmosphere in the chamber is kept moist by introducing a little water.

In order to allow diffusion of the gas from the chamber into the cell containing the suspended culture, the slide on which the culture is carried is pierced as already mentioned. The end of this slide is abutted against one of the plain slides used to seal the chamber and is pushed across the bars, thus replacing the blank slide, until the central perforation through the slide into the cell is over one of the openings of the central bar leading into the chamber. Free diffusion is thus established between the gas in the chamber and the atmosphere in the culture cell. The apparatus thus sealed and charged with gas is placed in an incubator at 39°C. The slight increase in pressure incident to the rise in temperature is relieved by momentarily opening one of the pinch clamps.

To remove the culture for examination the perforated slide is replaced by a blank slide in the same manner as described above. By this method the gas in the chamber is maintained without loss. This fact has been verified by analysis after each period of incubation.

In this study the gases used were: *a*, atmospheric air as control; *b*, 79 per cent carbon monoxide and 21 per cent oxygen; and *c*, 0.1 per cent illuminating gas in air. The carbon monoxide was prepared by the action of concentrated sulphuric acid upon formic acid at 80°C., and was washed repeatedly with caustic alkali solution and water to exclude possible extraneous irritant gases incident to its manufacture. The illuminating gas was the average run supplied to the City of New Haven. The local gas company has kindly furnished the following analysis: carbon dioxide, 3.5 per cent; benzol (C_6H_6), 1.0 per cent; olefines ($C_n H_{2n}$), 5.6 per cent; oxygen, 4.0 per cent; carbon monoxide, 21.4 per cent; hydrogen, 43.2 per cent; methane, 19.8 per cent; nitrogen, 5.1 per cent.

Digitized by Google

Examination was made of the tissue cultures after 24, 48 and in some cases 72 hours of exposure to the various atmospheres. In the accompanying table (page 245) are given the results obtained.

It is here to be seen that the percentages of successful growths in air (79 per cent nitrogen and 21 per cent oxygen) and in an atmosphere of the same oxygen tension but 79 per cent of carbon monoxide instead of nitrogen, were virtually the same. Indeed the latter, by the mere chance of experimentation, shows a slightly better percentage than the former—90.1 against 86.1. The growth observed with the atmosphere containing 79 per cent of carbon monoxide was in all respects as normal as that obtained when the gas in the cell was atmospheric air.

The fact that delicate neuroblast will propagate in an apparently normal manner when exposed to a tension of carbon monoxide so great as to be in the body physologically impossible, is conclusive evidence that carbon monoxide does not exert a specific toxic action upon nervous tissue.

Illuminating gas does, however, contain some substance toxic to nerve cells for no successful growths at all were obtained even in highly diluted gas (0.1 per cent). This is obviously due to some constituent of the gas other than carbon monoxide. But it is important to remember that the exposed tissue in a culture is probably more sensitive than the normal living animal or man. The fact that illuminating gas is more toxic than its content of carbon monoxide would wholly account for, has been noted previously in this laboratory (4), but one must not over-estimate this fact. It remains true that the toxicity of illuminating gas when inhaled is chiefly due to carbon monoxide; but in the body carbon monoxide acts through combination with hemoglobin and consequent asphyxia, and not specificially upon nerve tissue.

SUMMARY AND CONCLUSIONS

1. A technique is described whereby the growth of in vitro cultures of nervous tissue from the chick may be tested in any desired atmosphere or mixture of gases.

2. Carbon monoxide even in concentrations of 79 per cent is found to have no ill effect upon growing nerve cells. In this respect this gas is as neutral as nitrogen.

3. Illuminating gas has been studied in like manner and found to be toxic for neuroblast cultures in concentration of even as little as 0.1 per cent.

Digitized by Google

4. These observations indicate that carbon monoxide has no specific reaction with nerve tissue but acts in the body only through the asphyxia incident to its combination with hemoglobin.

5. These experiments indicate, also, that illuminating gas contains another toxic substance or substances; but this observation is not to be interpreted as weighing against the fact, well demonstrated, that asphyxia from inhalation of illuminating gas is chiefly due to carbon monoxide.

I wish to express my thanks to Prof. Yandell Henderson for advice and criticism.

BIBLIOGRAPHY

(1) Haldane: Journ. Physiol., 1895, xlix, 430.
(2) Harrison: Proc. Soc. Exper. Biol. and Med. 1907, iii, 162; Journ. Exper. Zoöl., 1910, ix, 287.
(3) Burrows: Journ. Exper. Zoöl., 1911, x, 63.
Carrell and Burrows: Journ. Amer. Med. Assoc., lvi, 116.
(4) Henderson and Haggard: Journ. Pharm. Exper. Therap., 1920, xvi, 11.

Digitized by Google

THE RELATION OF SPLENECTOMY TO GROWTH AND APPETITE IN THE RAT[1,2]

ARTHUR H. SMITH AND LEAH ASCHAM

From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

Received for publication December 9, 1921

Throughout the entire literature on splenectomy is repeatedly found the idea that increased appetite and decreased activity result from the operation. In Richet's (1) extensive experiments on the effects of splenectomy in dogs, he reported that after the operation the animals ate more than "normal" dogs did but gained slightly less in weight. When the splenectomized animals starved, they lost weight more rapidly than control animals under the same conditions and when they failed to continue to eat more than control dogs, they finally died of starvation. According to Richet, the splenectomized animals maintained a body temperature slightly higher than that of the controls and showed a very active metabolism. Danoff (2), moreover, concluded that although the respiratory quotient remained unchanged, there was an increase in carbon dioxide output and oxygen consumption after splenectomy. Richet used thirteen animals some of which he discarded for one reason or another when he drew his conclusions. The diet—a soup of bread, milk and water ad libitum and cooked horse meat fed separately—which was given by Richet, offers some difficulty in accurate measurement. The matter of normal and abnormal growth and the correlated food intake is one which must be subjected to statistical methods of study. Hitherto, in the case of the dog, these phenomena have not been measured in sufficiently numerous cases nor have the feeding conditions been carefully enough supervised to warrant drawing fine distinctions between normal and abnormal rates of growth. Moreover, unless dogs are reared in the laboratory on a definite feeding program, early habits, diversity

[1] Part of the expenses of the investigation was defrayed by a contribution from the Russell H. Chittenden Research Fund for Physiological Chemistry.

[2] This work was reported at the December (1921) meeting of the Society for Experimental Biology and Medicine, New York.

Digitized by Google

of previous treatment and variation in breed play a large part in their behavior under experimental conditions. Reports from the older literature that "the animals were increasingly voracious after splenectomy," etc., must be considered more as interesting observations than as scientific data. In the case of rats, Prym (3) stated that increased appetite was observed; but no data are available. Henn (4) likewise reported experiments on the growth of rats, kittens and puppies after splenectomy but the food intake of the animals was not carefully controlled.

It is desirable, therefore, to have more accurate information on the question of appetite and growth after splenectomy. The white rat is an animal admirably suited to experiments on food intake and growth, for these have been extensively studied and average curves for thousands of individuals are available. In view of the persistence of this idea of the relation of the spleen to appetite, we have studied the effect of splenectomy on the food intake and growth in the white rat.

The animals were splenectomized as nearly at the age of 40 days as possible. The simple operation was done under ether anesthesia and the splenic vessels were ligated *en bloc* with silk. Although the average growth and food intake curves of large numbers of individuals are available from other studies, it was thought desirable to run control animals under the same environmental conditions. In the control rats an abdominal incision was made and the spleen drawn out and replaced, the whole operation simulating as far as possible the splenectomy.

The rats were fed ad libitum upon the diet of purified materials described by Osborne and Mendel (5). The technic has been outlined by Ferry (6). Vitamin-B was provided by 0.4 gram dried brewery yeast daily, fed apart from the other food. The rats were weighed twice each week and their food intake was determined.

Of the first group of rats, five were observed for 34 weeks after splenectomy; three were observed for 43 weeks. All of these animals grew normally or above for about the first 140 days. In no case was this normal growth accompanied by consistently increased food intake. Usually our splenectomized rats ate slightly less food than indicated by the curves for the food intake of normal rats.

From about the 140th day on, a marked change occurred in growth rate of the male rats of both the experimental and the control groups. The increments in body weight were smaller than expected and continued so until, at the end of the experiment at the age of 240 days, the male rats were from 50 to 75 grams below the normal average weight for this age according to Osborne and Mendel (7). Rats 8 and 10, both

Digitized by Google

splenectomized males, were exceptions in that they continued growing at the normal rate for 210 days before they declined. In both these animals the food intake after the 200th day was slightly above that of our controls. Unfortunately, average normal food curves were unobtainable for this advanced age in rats.

Better growth rate was observed with the female rats. Three of the four splenectomized females grew at a rate above normal during their entire life. The fourth (rat 4) declined very slowly from the 160th day being, however, only 25 grams below the average normal weight at the 240th day. None of these splenectomized females ate appreciably more food than did the controls nor more than that indicated by the average food intake of normal animals on the same ration.

Rats 7 and 15, two of the splenectomized females, were mated with a splenecotmized male (rat 8) and raised litters. The young were apparently normal. Rat 15 raised seven of a litter of eight and rat 7 raised three of a litter of eight, the remainder being killed because of poor condition. Eight of these "second generation" rats were splenectomized and their growth and food consumption likewise observed for 160 days. Here again all of these animals grew at a rate equal to or slightly greater than that indicated by the normal average curves. The rate of food intake was equal to or very slightly less than what was expected.

From these observations on splenectomized rats and on their splenectomized progeny, *there is no indication whatever of an increased appetite as a result of the removal of the spleen.* The animals grew at the same rate on the same food intake as did the control rats, until the 140th day, as was indicated by the average normal curves for growth and food intake. The data obtained from these experiments seem to present conclusive evidence against the idea that the presence or absence of the spleen plays a part in affecting the nutritive requirements of the white rat. These results on the nutrition of the rat after splenectomy harmonize with those of Goldschmidt and Pearce (8) and of Paton (9) who studied the metabolism of the dog after splenectomy.

Among the many functions attributed to the spleen has been the one of altering the blood. In the older literature there are many conflicting results on the blood after splenectomy, anemia being reported in some cases and not in others. The extensive work of Pearce (10) and his associates on splenectomized dogs seems to point to a definite anemia of varying degrees of severity and of varying duration. Furthermore, the increased resistance of the erythrocytes of splenctomized animals

Digitized by Google

to hemolysis by hypotonic solutions has been reported repeatedly. On the other hand, Paton, Gulland and Fowler (11), working on dogs, cats and rabbits, found no effect on the blood picture following splenectomy. Wolferth (12) reported a slight transient anemia following the splenectomy in white rats.

Erythrocyte counts were made in five of our splenectomized rats whose parents had likewise been splenectomized. The end of the tail was clipped and the blood sample was taken when the blood oozed out freely. As will be seen from the table, counts were made before splenectomy, 4 to 7 days after splenectomy, a week later, and then every 4 days until 7 weeks after splenectomy. It was hoped thus to detect blood changes occurring within 2 weeks and especially those occurring from 3 to 6 weeks after splenectomy at which time, according to Pearce, the anemia in dogs reaches its maximum severity. The values for the red cell count are somewhat variable, but in no case was there an unmistakable anemia after removal of the spleen. There was a mere suggestion of a decrease in rats 25 and 23 but even in these animals the effect was transient and of small magnitude.

TABLE 1

RAT 19		RAT 20		RAT 23		RAT 24		RAT 25	
Days after splenectomy	Red count	Days after splenectomy	Red count	Days after splenectomy	Red count	Days after splenectomy	Red count	Days after splenectomy	Red count
	6,980,000		6,180,000		8,530,000		8,370,000		8,290,000
8	7,080,000	8	6,450,000	4	7,800,000	4	8,630,000	4	7,430,000
11	7,470,000	11	7,150,000	9	7,670,000	9	7,600,000	9	7,300,000
16	7,350,000	16	7,200,000	16	8,500,000	16	8,640,000	16	9,000,000
21	7,800,000	21	7,680,000	19	8,390,000	19	7,910,000	19	7,410,000
28	7,300,000	28	7,710,000	23	8,420,000	23	7,560,000	23	8,370,000
31		31	8,160,000	26	8,640,000	26		26	7,660,000
35	8,510,000	35	8,250,000	30	8,850,000	30	7,800,000	30	8,190,000
38	7,640,000	38	7,180,000	33	8,310,000	33	8,370,000	33	8,480,000
42	8,180,000	42	7,560,000	37	8,860,000	37	8,250,000	37	8,970,000
45	7,870,000	45	8,030,000	41	8,500,000	41	8,430,000	41	8,780,000
49	7,930,000	49	7,670,000	45	8,230,000	45	8,020,000	45	8,860,000
52	7,920,000	52	7,820,000	48	8,900,000	48	8,500,000	48	8,950,000
56	8,050,000	56	7,770,000						
59	8,050,000	59	7,830,000						

Digitized by Google

In experiments conducted in this laboratory, Geiling and Green (13) reported that, after hemorrhage, splenectomized rats regenerate their blood in normal time. These facts taken together with our observations make it appear doubtful that removal of the spleen in the white rat results in changes of appreciable magnitude in the erythrocyte count.

Rats 7, 10, and 15 were sent to Dr. Donaldson of The Wistar Institute who kindly examined them for any structural changes resulting from splenectomy. The organs and bones were normal in weight and measurement and showed no effect of splenectomy. The marrow of the bones of rats 7 and 10, animals which had been bled small amounts for blood counts and hemoglobin estimations for a short time after splenectomy, showed the expected change from yellow to red while the bones of rat 15 did not show this change.

SUMMARY

A study of the body weight and food intake of the white rat after splenectomy gave no evidence of an increase in appetite or variation from the normal rate of growth.

A study of the red cell count on five splenectomized rats which were the progeny of splenectomized parents showed no anemia followed the removal of the spleen.

BIBLIOGRAPHY

(1) Richet: Journ. d. Physiol. et d. Pathol., 1912, xiv, 689; 1913, xv, 579.
(2) Danoff: Inaug. Diss., Univ. Bern, 1918, 23.
(3) Prym: Verhand d. Kongr. f. innere Med., 1911, xxviii, 398.
(4) Henn: This Journal, 1920, lii, 562.
(5) Osborne and Mendel: Journ. Biol. Chem., 1920, xli, 517.
(6) Ferry: Journ. Lab. Clin. Med., 1920, v, 2.
(7) Donaldson: The rat, 112.
(8) Goldschmidt and Pearce: Journ. Exper. Med., 1915, xxii, 319.
(9) Paton: Journ. Physiol., 1900, xxv, 443.
(10) Pearce, Krumbhaar, Frazier: The spleen and anemia.
(11) Paton, Gulland and Fowler: Journ. Physiol., 1902, xxviii, 83.
(12) Wolferth: Arch. Int. Med., 1917, xix, 105.
(13) Geiling and Green: Proc. Soc. Exper. Biol. and Med., 1921, xviii, 191.

Digitized by Google

THE RELATION OF THE ADRENALS TO FATIGUE[1]

F. A. HARTMAN, R. H. WAITE AND E. F. POWELL

From the Department of Physiology of the University of Buffalo

Received for publication December 15, 1921

Experimental work of recent years has indicated that the adrenal medulla is not essential to life (1). Operations in which all of the medulla of a single remaining adrenal has been destroyed have produced no specific symptoms.

Although it has been shown experimentally that epinephrin, the secretion of the medulla, can modify the activity of many tissues in the body, the question as to how much this takes place normally is unanswered.

Stewart and Rogoff (2) have been unable to show that a reduction of the epinephrin output to $\frac{1}{800}$ of the normal or less produces any difference in the animal. In view of the very suggestive emergency theory of Cannon (3) it would be interesting to know whether such animals as those just mentioned could meet conditions of stress as well as normal animals. So far as we know the animals of Stewart and Rogoff were permitted to lead a quiet life as most laboratory animals do.

We have tested the ability to work and to withstand fatigue, and in one instance the effect of pregnancy, in animals with a reduced epinephrin output.

We have obtained evidence that animals, with but one adrenal and that denervated, do not possess the endurance of normal animals. Our results with the denervated pupil suggest that this is due to an insufficient supply of epinephrin.

Care of animals. Many of our animals were observed for some time and were tested before the operation. In most instances the removal of one adrenal and denervation of the other took place at one operation, the abdominal path being used. Every possible precaution was taken to prevent infection, the regular hospital routine being followed. The animal was carefully protected by sterile cotton and an abdominal

[1] This study was aided by a grant from the Research Fund of the American Association for the Advancement of Science.

Digitized by Google

bandage in the form of a jacket with openings for the four feet. Primary infections were absent. If secondary infection occurred, which was uncommon, the experiment was discontinued except where the infection yielded readily to antiseptic treatment.

The animals were fed with boiled liver, milk or bread and milk, occasionally table scraps and yeast at intervals. Even kittens thrived upon this diet.

They were housed in a large, well-lighted room connecting with a large outdoor enclosure. They were permitted to roam at will through this room and enclosure.

Treadmill for fatigue. The apparatus for fatiguing animals consisted of a wooden treadmill with an inside diameter of 31 inches and an inside width of 11 inches. The treading surface was composed of slats placed far enough apart for ventilation. The sides were covered internally with transparent celluloid to keep the animal from clinging to the wire gauze. Access to the inside was by means of a door in the circumference. It was arranged so that at each revolution of the mill an electrical contact was made. A signal magnet, for a kymograph record, and an electrical counter were connected in the circuit. In some instances an electric fan was directed toward the tread in order to increase the ventilation.

The mill was turned by means of a crank so that the operator could vary the rate or change the direction to suit the whim of the animal or the condition of fatigue. It was found from experience that by carefully watching the animal and varying the speed to accommodate it and also changing the direction when it appeared tired of pacing, much more work could be obtained. With practice it is possible to induce many cats to work, which ordinarily refuse to work in the mill.

There are cats which never work satisfactorily in the treadmill either from clumsiness or obstinacy. This must be considered in the tests.

The treadmill method was found to be more satisfactory than leading an animal around the room because when the animal becomes slightly fatigued or is disinclined to work he permits himself to be dragged and does little work. In the treadmill the stimulus for work is greater and much more work is done in a unit of time than in walking on the level.

Observations. A period of ill health greater than that in controls sometimes follows removal of one adrenal and denervation of the other. This stage is characterized by loss of appetite, loss of weight, change in behavior and in some cases a lowered temperature. Diarrhea is common

Digitized by Google

but seems to be due to cutting the splanchnic nerve, for it is produced in operations where only the splanchnic nerve is cut. The following gives briefly a comparison of denervated adrenal animals and control animals.

CAT NUMBER	WEIGHT LOST	DAYS TO REGAIN WEIGHT FROM TIME OF OPERATION	LOWEST TEMPERATURE RECTAL
One adrenal removed, other denervated			
101	17% in 13 days	35 to regain all	Normal
103	29% in 16 days	28 to regain all	Normal
104	36% in 29 days	36 to regain 7% (accidently killed)	36.6° C. at 29 days normal at 37 days
105	21% in 12 days	60 to regain 5%	37° C. at 14 days normal at 19 days
111	14.3% in 15 days	41 to regain 11.6%	Normal
One semilunar ganglion removed			
102	6.0% in 9 days	12 to regain all	Normal
One semilunar ganglion removed opposite adrenal manipulated			
108	12.0% in 4 days	14 to regain all	Normal
One adrenal removed, adrenal vein tied on other side			
109	10% in 30 days	47 to regain all	Normal
Semilunar ganglion dissected free except from splanchnic and capped with rubber			
123	6.8% in 20 days		

Although it would require a large number of animals to reach a definite conclusion our animals from which one adrenal had been removed and in which the nerves to the other gland had been cut, certainly were affected more than the controls. The greater loss in weight, subnormal temperature and change in behavior might be accounted for by the decreased adrenal function, the recovery, by regeneration of the nerves and a consequent increase in function.

The ability of all animals to work and to withstand fatigue has been tested by the treadmill. In our later work we used the denervated eye as a possible test for epinephrin in the work tests. The following evidence indicates that this test can be used for epinephrin output in fatigue. This is based upon the most reasonable interpretation of our observations which follow.

Digitized by Google

The denervated eye reaction as a test for epinephrin in fatigue. If an animal, from which one of the superior cervical ganglia has been removed, is caused to work in a treadmill, after a few minutes the denervated pupil becomes larger than the control pupil. This might be due to inhibition of the tonic activity of the ciliary nerve or it might be due to the action of an increased output of epinephrin from the adrenals. Meltzer and Auer (20) have shown that the sensitivity of the pupil reaction to epinephrin is increased very much above normal by previous removal of the superior cervical ganglion.

In some animals the response of the denervated eye develops more easily than in others. We chose a cat in which there was always a good dilatation of the denervated pupil as compared to the control, within two or three minutes after beginning the work in the treadmill. This dilatation continued throughout the test. The right adrenal had been removed twenty-five days previously. The remaining adrenal was removed through as small an opening as possible by the lumbar path. On the morning following this operation, although the cat worked for one hour in the treadmill, travelling 728 m., no dilatation of the denervated pupil occurred throughout the experiment, this pupil being smaller than the control at all times as in a condition of rest. In the afternoon of the same day a further test of 430 m. failed to produce a positive pupil reaction. Yet previous to the removal of the second adrenal a good dilatation of the denervated pupil occurred invariably within two to three minutes and fifty or more meters. Apparently taking out the second adrenal removed the cause of dilatation of the denervated pupil which occurs during muscular activity. On the other hand the denervated pupil could be made to dilate by painful stimulation (tetanizing current applied to saline soaked ear) or by partial asphyxia (occlusion of trachea).

In another cat which usually gave a good dilatation of the denervated pupil beginning five minutes after the work in the mill started and gradually increasing throughout the test, after the removal of one adrenal and denervation of the other, repeated tests failed to give the denervated pupil reaction. However, one hundred and sixty-eight days after the operation the fatigue test again elicited dilatation of the denervated pupil although the reaction developed more slowly and less intensively than before. The adrenal was again exposed and found to be connected with nerve fibers from the splanchnic nerve. These were destroyed. Later repeated fatigue tests failed to elicit dilatation of the denervated pupil.

Digitized by Google

Knowing the affect of epinephrin upon the denervated pupil it is most reasonable to suppose that the dilatation of the denervated pupil during work is due at least in large part to an increase in the epinephrin output.

Relation of the denervated pupil reaction to the work performed. A cat which shows a good dilatation of the denervated pupil accompanying work in the treadmill is able to do more work than it can when such dilatation is absent.

Although dilatation of the denervated pupil occurred with great difficulty in cat 101, bursts of fast work were usually accompanied by dilatation of the denervated pupil. Moreover the animal was able to travel only 728 m. when there was no accompanying dilatation of the denervated pupil, while on the same day when a certain amount of denervated pupil dilatation accompanied the test, the animal travelled 1438 m.

Cat 103 likewise was able to travel only 855 m. when no dilatation of the denervated pupil accompanied the test, but in the afternoon of the same day was able to go 1800 m. when there was some dilatation of the denervated pupil. In a similar manner spurts of work were attended by increases in the dilatation of the denervated pupil. This animal later (two hundred and fifty-seven days after cutting nerves to adrenal) recovered the denervated pupil response so that it was easily elicited in the treadmill. The farther it travelled the greater became the dilatation. At this time it was able to travel 3720 m. in the treadmill and there was no sign of convulsions, in fact it could have gone farther. This striking change can only be accounted for by a regeneration of the nerves to the adrenal.

Whenever Cat 105 worked well in the treadmill the denervated pupil became dilated, especially accompanying spurts of work. From the time that the animal was first tested as to the pupil reaction (fifty-three days after removal of one adrenal and cutting the nerves to the other) the denervated pupil always showed some response which we thought might be explained by regeneration of the nerve fibers. At autopsy (sixty-one days after adrenal operation) this appeared to be the case because nerve fibers again connected the semilunar ganglion with the adrenal.

Cat 111 also demonstrates the intimate association of work power with adrenal activity. At first when the adrenals were intact every test, except one, elicited a good pupil reaction. In this one test the pupil reaction was entirely absent. The cat was able to travel only

Digitized by Google

741 m. because of the appearance of convulsions. But on the afternoon of the same day in which this test was made, the cat when tried in the treadmill gave an excellent pupil dilatation and travelled 2240 m. Even that was not the limit of possible work. The denervated pupil dilated more and more as the work progressed until the stage of marked dilatation was reached. Spurts of work were attended by increases in the dilatation. Soon after removal of the right adrenal and of the left semilunar ganglion, the cat refused to work well at any time, likewise dilatation of the denervated pupil was absent. However, one hundred and sixty-eight days after the latter operation work in the treadmill was accompanied by moderate dilatation of the denervated pupil. The accompanying work was an improvement but not so good as that obtained before the adrenal operation.

The remaining adrenal was again exposed. Nerves had regenerated from the splanchnic to the adrenal. These were cut. Tested later, the animal refused to work well although sufficiently to elicit a good dilatation of the pupil under normal conditions, yet dilatation was entirely absent.

In another cat which possessed but one adrenal and that denervated, a good eye reaction was obtained accompanying work. At autopsy, nerve fibers were found connecting the splanchnic with the adrenal.

Cats possessing at least one normal adrenal give dilatation of the denervated pupil after working in the treadmill a short time. This was true for 13 normal animals which we tested. In only two cats, one instance in each case, did such dilatation fail to appear. However these cats when tested at other times gave good dilatation of the denervated pupil.

Denervated eye reaction in convulsions caused by fatigue. Most cats, which work hard in the treadmill and at the same time give a good denervated pupil dilatation, show no ill effects beyond a marked appearance of fatigue even if they travel far. On the other hand when the denervated pupil fails to dilate during the development of fatigue, if the animal is required to travel far, convulsions often occur. At least these are the results in our cases.

In cats with intact adrenals fatigue convulsions are difficult to obtain. One good example of such a case however is found in our cat 111 in which both adrenals were intact. On March 19, at 12 m. the cat travelled 780 m. in forty-one minutes giving a good dilatation of the denervated pupil. At 3 p.m. of the same day a ten minute test of 111 m. likewise produced a good denervated pupil dilatation.

Digitized by Google

Two days later at 9 a.m. the cat was required to travel 741 m. in fifty-one minutes, the test being terminated by convulsions. There was no dilatation of the denervated pupil throughout the test. Seven hours later, on being tested in the treadmill, the denervated pupil began to dilate after the first few minutes of work, becoming larger and larger as time went on. At fifty-six minutes there was a very active period in which the animal paced very rapidly for many revolutions. This was accompanied by a still greater dilatation of the denervated pupil. The animal continued to work until it had travelled 2240 m. in two hours, the denervated pupil being extremely dilated at that time. The cat was now weak but could have continued its work longer. This animal travelled faster and three times farther when there was a good dilatation of the pupil as compared to the test with absence of denervated pupil dilatation. Moreover convulsions appeared in the latter but were entirely absent in the former test. Repeated attempts have not been able to produce convulsions in this animal since the time mentioned above.

In some cats with but a single adrenal, and that denervated, convulsions were very easily produced by fatigue. (See protocols for cats 101 and 103.) In cat 101, five out of eighteen tests ended in convulsions and in only one of these, the last, was there any evidence of dilatation of the denervated pupil (i.e., so that the denervated pupil was larger than the control). This last test was made two hundred and seventy days after the primary adrenal operation, allowing ample time for regeneration of nerve fibers and perhaps accounting for the dilatation which did occur. This dilatation, however, was very tardy and not very marked.

In cat 103, out of six tests within sixty-four days following the adrenal operation, three were terminated by convulsions. In none of the three was there dilatation of the denervated pupil. Yet two hundred and five days later, this cat travelled 3720 m. in one test without showing any evidences of convulsions. This is nine times as far as it usually travelled in the tests during the period when convulsions were easily produced. This test was accompanied by a very marked dilatation of the denervated pupil. Ample time for the regeneration of nerve fibers had elapsed.

All cats with a single adrenal and that denervated do not give convulsions upon testing in the treadmill. Some lack aggressiveness to the extent that they cannot be made by any means to work hard. If the animal does not work hard the conditions which produce convul-

Digitized by Google

sions never develop. This is true even in the entire absence of adrenals. Here the animal which we have tried lacks the staying power to carry it to the point of fatigue where convulsions might develop. Our observations lead us to the conclusion that fatigue accompanied by well marked dilatation of the denervated pupil is scarcely ever followed by convulsions.

Fatigue convulsions. The convulsions which resulted from work in the treadmill were caused by fatigue and not by dizziness. As proof that the latter was not a factor, cat 103, at the period when convulsions were easily produced, was tied to the tread of the mill, the mill then being rotated. The cat with head free was enclosed by a bag so that it could obtain the visual effects of the movement. The cat was rotated 1250 m. in seventy-one minutes. At no time was there any sign of discomfort, and there was never an indication of convulsions. We have conclusive evidence as to the cause of these convulsions, in the fact that they are never obtained except following hard work.

Although fatigue appears to be the cause, we do not know the manner in which this brings about convulsions. Perhaps fatigue products play a part in their onset because even though you stop the work immediately upon the first sign of twitching, the convulsions develop to their maximal extent. (See protocol, cat 103.) Or it might be a more purely nervous phenenomon, with fatigue products as accessory factors.

The usual course of events is as follows: The cat begins to twitch and jerk in a few muscles; this activity spreads and the intensity increases until virtually the whole musculature undergoes spasmodic contraction, the animal lying on its side. There is also marked salivation, maximal dilatation of both pupils and dyspnea accompanying the later stage. The duration of the convulsions is usually about ninety seconds. Convulsions are followed by nervousness in some cats but with little apparent change in others except the prostration of fatigue. No ill effects seemed to result in any instance.

We have been able to produce these convulsions in but two normals and only once in each. Two other cats showed convulsive movements but not the typical convulsions with prostration. In all we have tested 19 normal animals. In the two instances of convulsions the denervated pupil was smaller than the control up to the appearance of convulsions. These two cats gave good dilatation of the denervated pupil in other tests and there were no convulsions.

Thirteen of the 19 normal animals had the superior cervical ganglion removed on one side. During work in the treadmill all of these gave

Digitized by Google

greater dilatation of the denervated pupil than the control except in the two instances above mentioned.

Of five animals which possessed but a single adrenal, and that denervated, two (no. 101 and no. 103) gave little or no dilatation of the denervated pupil when worked in the mill and in these convulsions could be obtained repeatedly by fatiguing in the mill. Convulsions were never caused in the other three, one always responded by a good denervated pupil reaction while the other two refused to work very hard. One of the latter also gave a fair denervated pupil reaction at times, while the second did not have a denervated pupil.

Because convulsions appeared only when there had been little evidence of epinephrin preceding them and because they appeared to be due to something suddenly released into the system, we have attempted to produce convulsions in animals by the injection of toxic doses of epinephrin.

Lethal doses of adrenalin were injected into two kittens and one adult cat but no evidence of convulsions appeared in any one of these.

Two guinea pigs were tried as follows:

Protocol, guinea pig, 695 grams

a.m.
10:25. Injected 0.1 cc. 1:1000 adrenalin into ear vein.
10:31. Injected 0.1 cc. 1:1000 adrenalin into ear vein.
10:34. Injected 0.15 cc. 1:1000 adrenalin into ear vein.
10:36. Spasmodic breathing, gasping but no convulsions.
10:39. Still breathing in gasps.
10:41. Died. No convulsions.

Protocol, guinea pig 700 grams

10:49. Injected 0.1 cc. 1:1000 adrenalin into ear vein.
10:50. Begins to quiver.
10:54. Does not move quickly when touched.
11:06. 0.1 cc. 1:1000 adrenalin injected into ear vein.
11:07. Lies down.
11:09. Dyspnea.
11:23. Restless.
11:25. Few spasmodic contractions of whole body.
11:28. Spasmodic contractions again.
11:30. Spasms more pronounced.
11:31. Urinated.
11:35. Spasmodic contractions, which recurred at intervals of 2 or 3 minutes until they become quite severe.
p.m.
12:08. Spasms less frequent and less severe.
12:33. Spasms still appearing.

Animal recovered.

Digitized by Google

On the next day, 3 intravenous injections (1:1000 adrenalin) of 0.1 cc. each given at 11:36, 11:41 and 11:43 a.m., respectively, failed to produce spasms. It seems impossible that epinephrin might be suddenly increased to this extent in animals having but a single, more or less denervated adrenal. Likewise epinephrin convulsions seem to be rarely obtained.

Protocol, cat 101. After having kept this animal under observation for one month, the right adrenal and the left semilunar ganglion were removed by the lumbar route. Before the operation, tests were carried out in the treadmill but not to the point of great fatigue, e.g.., in one test 370 m. were covered in 5.5 minutes, in another 411 m. in 10 minutes.

17 days after operation. Wound healed, cat travelled 332 m. in 6 minutes. Tired.

18 days after operation. Travelled 180 m. in 5.5 minutes.

19 days after operation. Travelled 358 m. in 12 minutes; so completely fatigued that it could neither keep pace nor hold on to the tread.

20 days after operation. Travelled 488 m. in 17 minutes.

21 days after operation. Travelled a total of 525 m. in two work periods. Approximately equal in time (5 minutes) and distance but 30 minutes rest between.

23 days after operation. Travelled 1590 m. in three periods of about 19 minutes each and one had 4 hours rest periods between.

25 days after operation. Travelled 515 m. in 10 minutes after 30 minutes rest, travelled an additional 515 m. in 16 minutes. The second period was terminated by *convulsions* following which the cat raced around the room four times and then relaxed, tremors persisting in the forelegs.

27 days after operation. Travelled 528 m. in 18 minutes.

30 days after operation. Travelled 764 m. in 16 minutes *convulsions* terminating the test.

32 days after operation. Travelled 755 m. in 18 minutes.

47 days after operation. Travelled 423 m. in 6 minutes. Two hours later travelled 545 m. in 15 minutes. Seven hours after first test, cat travelled 473 m. in 28 minutes, *convulsions* at close.

52 days after operation 1, removed the left superior cervical ganglion.

65 days after primary operation again tested in the mill. At 9 a.m. after 21 minutes, 660 m. the cat held weakly to the slats being unwilling to make the effort of pacing.

At 26 minutes, 728 m. very marked convulsions which lasted 90 seconds. After convulsions had ceased the cat whined and was extremely irritable so that if disturbed she jumped about irrationally. When undisturbed she showed spasmodic jerks. There was no dilatation of the denervated pupil up to the time of the convulsions.

At 3:30 p.m. cat again in treadmill. This time the denervated pupil occasionally dilated to the size of the control pupil. The animal after 16 minutes, 302 m., could do nothing but slide and hold feebly to the slats until 1 hour, 1300 m., when there was a burst of working power, the cat pacing 138 m. very rapidly without cessation. Following this the denervated pupil was larger than the control. Test stopped at 70 minutes, the animal refusing to work further.

Digitized by Google

Seventy-six days after primary adrenal operation, adrenal and lumbar veins to the remaining adrenal, tied.

This cat became pregnant, six healthy kittens being born one hundred and eighty-two days after the primary operation.

Two hundred and seventy days after primary adrenal operation. Tested in the treadmill. After 5 minutes, 155 m., denervated pupil still smaller than the control. After 10 minutes, 333 m., denervated pupil just larger than the control. At 843 m. there was a spurt of fast pacing continuing for 104 m. when the animal was seized with convulsions. Pupil reaction was very slow to develop. The animal did not appear to be as aggressive as it had formerly. It really worked well only at the last spurt.

Protocol, cat 103. Removed right adrenal, cut nerves to left adrenal. Forty-three days later, destroyed left superior cervical ganglion. Fifty-seven days after adrenal operation, tested in treadmill. Paced practically all of the way, going 476 m. in 14 minutes. There was no dilatation of the denervated pupil throughout until the convulsions which terminated the test. Convulsions were accompanied by urination and defecation. Animal very irritable afterward.

Fifty-eight days after primary operation. *Tested at 8:25 a.m.* Worked hard in the mill; tried to avoid pacing but did slide. No dilatation of denervated pupil. Convulsions at 855 m., 27 minutes.

Tested at 4 p.m. Weak, hangs to tread and drops or jumps much of the time. No dilatation of denervated pupil until 80 minutes, 1661 m. when there was a spurt of fast pacing (111 m. in 2 minutes). This was accompanied by enlargement of the denervated pupil to an equality with the control. Spurts of work occurred from time to time after this, being accompanied by a similar dilatation of the denervated pupil. Stopped at 96 minutes, 1800 m., the cat raced wildly about the room and then lay down. Tested the next morning the cat refused to work hard at any time. There was no dilatation of the denervated pupil.

Sixty-four days after primary operation. Tested in treadmill for 33 minutes, going 1020 m. No dilatation of denervated pupil throughout the experiment, which was terminated by convulsions lasting 85 seconds. Very nervous afterwards. The treadmill was being turned very slowly and was instantly stopped at the first sign of twitching. In spite of this, the twitches and jerks became gradually more severe until violent convulsions had developed. It seemed that once the threshold was crossed convulsions developed.

Two hundred fifty-seven days after primary operations. Tested in treadmill, denervated pupil larger than control at 6 minutes, 136 m. At 17 minutes, 370 m., denervated pupil very much larger than control. At 27 minutes pacing unusually fast and well ("second wind"). At 35 minutes, 1000 m., cat still travelling.

Two hundred sixty-nine days. Tested in treadmill.

After 3 minutes, 87 m., denervated pupil just larger than control.

At 20 minutes, 690 m., denervated pupil just larger than control.

At 35 minutes, 1120 m., denervated pupil decidedly larger than control.

At 83 minutes, 2600 m., denervated pupil much larger.

At 120 minutes, 3500 m., denervated pupil very much larger.

At 131 minutes, 3720 m., denervated pupil very much larger than control.

Although the animal was tired it could have travelled farther.

Digitized by Google

Discussion. In experiments of long duration, dietary factors must be carefully ruled out by feeding a variety of foods to assure a plentiful supply of the different vitamines. For example, we found that boiled liver and whole cow's milk lacked something for the proper growth of kittens, although adult cats apparently thrived on this diet. The addition of yeast made the diet adequate for kittens and was therefore used in the adults.

Occasionally mange gets a start among the cats and is readily spread. This can be checked by daily soaking of the hair and skin with a saturated solution of chloramine-T (4) on the ears, head and neck. Mange in the cat is first detected by the presence of tiny lumps in the skin on top of the head or neck or sometimes on top of the ears.

We also found it advisable to remove fleas from cats newly arrived at the laboratory by giving them a good soaking in a dilute solution of "Coronoleum" (West Disinfecting Company). By thoroughly drying the animal immediately afterward most of the "Coronoleum" is evaporated and the animals are less likely to be poisoned by licking the fur.

Our results indicate that the dilatation of a denervated pupil can be used as a test of increased adrenal secretion in muscular work, for removal of both adrenals or cutting the nervous connection with a single remaining adrenal abolishes the dilatation which accompanies muscular work. On the other hand such dilatation again reappears after regeneration of the nerve fibers.

Removal of one adrenal and cutting the nerves to the other as shown first by Elliott (5) and later by Stewart and Rogoff (2) certainly has shown that central nervous control of the adrenal is not necessary to maintain the vital processes. But our observations seem to indicate that this nervous control bears an important relation to the muscular efficiency of the animal. Tests of this must be made before the nerve fibers have time to regenerate. For if the nerves are cut in the abdominal cavity of the cat the central end has but a short distance to grow to reach the adrenal. It has been shown by Langley (6) that nerve fibers in the cervical sympathetic regenerate at a fairly rapid rate. If this rate holds for the splanchnic it should not take many days for some of the fibers to reach the adrenal, the distance is so short. That such regeneration does take place is indicated by our observations on the return of the denervated eye reaction to fatigue. Examination of our animals, in which we have suspected nerve regeneration, has shown that nerve fibers have again connected with the adrenal. Cut-

Digitized by Google

ting these fibers has again removed the nervous control of the adrenal as indicated by the denervated eye reaction in fatigue.

Of course many fibers no doubt fail to find their way back or do so after a very long interval so that the nervous control would be completed only after a prolonged period. However those that do begin to function are factors which must be considered in adrenal activity.

We are yet in the dark as to the nature of fatigue convulsions in cats. Because of the fact that these can occur in normal cats and that they do not occur in cats with a single denervated adrenal until some time after cutting the nerves (such a time as might permit regeneration of some nerve fibers), the rather remote possibility that they might be due to a sudden release of epinephrin suggested itself. In reviewing the literature covering the toxic effects of epinephrin we have found that epinephrin convulsions have been described by Batelli (7) and Schaller (8). The former says that they are frequent in animals before death from epinephrin. The latter said that he frequently produced typical epileptic attacks in rabbits from maximal sub-lethal injections of adrenalin.

On the other hand Foà and Pellacani (9), Oliver and Schäfer (10) and Vincent (11) described dyspnea, circulatory effects and prostration as the typical results following toxic doses of adrenal extracts. We have also found convulsions rarely produced by epinephrin injections.

Furthermore it is difficult to conceive of an arrangement which would permit a sudden flooding of the circulation with epinephrin. It is true up to the time of fatigue convulsions in our experiments, epinephrin is not poured into the circulation in sufficient amounts to affect the denervated pupil to any extent. On the other hand whenever the denervated pupil gives an indication of a steady outpouring of an increased supply of epinephrin, fatigue convulsions usually have not occurred.

Fatigue convulsions never develop unless the animal works hard, at least for a short time. Thus we have been unable to produce them by leading a susceptible cat (no. 103) around the room for 3400 m. (the cat did become tired but not greatly fatigued). The work performed in this test is a great deal less than that performed over a much smaller distance in the treadmill for the cat was supported to a considerable extent by a harness around the shoulders, otherwise it refused to travel. Likewise we were not able to produce convulsions in a cat from which both adrenals had been removed. This cat, however, did not work hard.

Digitized by Google

Observations from many sources point to a close relationship between the adrenals and muscular activity. First, the muscular weakness of adrenal insufficiency suggests an intimacy between the two tissues; second, the vacuolization of the adrenals after prolonged muscular activity (12); third, the size of the adrenals increases with the development of the muscles (13), (14).

A great deal of work has been done which shows that epinephrin may be of importance in muscular fatigue. The quantity of epinephrin in the suprarenals is very much decreased by great muscular fatigue (15). This has a great deal of significance when connected with the observation that epinephrin delays the onset of fatigue in skeletal muscle (16), (17), (18).

The beneficial effects of epinephrin in fatigue may be due not only to an increase in the circulation (19) to the muscle but to some other action yet undetermined (18).

Our observations furnish evidence of the increase of epinephrin during fatigue in normal unanesthetized animals. They show further that the absence of such an increase reduces muscular efficiency.

We wish to thank C. A. Mietus, E. D. Pillion, M. G. Potter and W. P. Taylor for their valuable assistance in this research.

SUMMARY

1. Normal cats usually give a dilatation of the denervated pupil accompanying work in a treadmill. This begins within a few minutes and increases as the work progresses. Spurts of work are accompanied by greater increases in the dilatation; the same cat will work harder and travel farther when such dilatation is present. If dilatation is absent and the animal works hard convulsions may follow but they usually do not result if there is a good dilatation of the denervated pupil.

2. The dilatation of the denervated pupil accompanying fatigue is absent in animals deprived of both adrenals or possessing but a single adrenal and that completely denervated. Such dilatation, therefore, is probably caused by epinephrin.

3. Cats possessing but a single adrenal, and that denervated, undergo a period of ill health which appears to be more marked than that of control animals. This is indicated by the loss of appetite, loss of weight, change of temperament, weakness and in some instances a subnormal temperature. During this period the working power is very much decreased. On the other hand, after regeneration of some of the nerve fibers, health and power to work are regained.

Digitized by Google

4. Certain cats possessing but a single adrenal, and that denervated, developed convulsions in the treadmill repeatedly. Preceding these, dilatation of the denervated pupil was scanty or absent. In the cat which developed these convulsions most easily, they could be obtained with difficulty after regeneration of a large number of the nerve fibers to the adrenal judging from the denervated pupil reaction. At this time the animal could travel many times farther in the treadmill than before regeneration of the nerves.

5. Our results seem to indicate that epinephrin plays a very important rôle in increasing muscular work and delaying the onset of fatigue.

BIBLIOGRAPHY

(1) Wheeler and Vincent: Trans. Roy. Soc. Canada, 1917, xi, 125.
(2) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, x, 1.
(3) Cannon: Bodily changes in pain, hunger,fear and rage, 1915, New York.
(4) Smith: Science, 1921, liv, 378.
(5) Elliott: Journ. Physiol., 1912, xliv, 374.
(6) Langley: Journ. Physiol., 1897, xxii, 215.
(7) Batelli: Compt. rend. Soc. de biol., 1902, liv, 1247.
(8) Schaller, quoted in Fisher: Journ. Nerv. Mental Dis., 1918, xlvii, 40.
(9) Foà and Pellacani: Arch. per le sci. med., 1884, vii, 9.
(10) Oliver and Schäfer: Journ. Physiol., 1895, xviii, 230.
(11) Vincent: Journ. Physiol., 1897, xxii, 111.
(12) Bernard and Bigart: Compt. rend. Soc. de biol., 1902, liv, 1400.
(13) Elliott and Tuckett: Journ. Physiol., 1906, xxxiv, 332.
(14) Watson: Journ. Physiol., 1907, xxxv, xlix.
(15) Batelli and Boatta: Compt. rend. Soc. de biol., 1902, liv, 1203.
(16) Panella: Arch. ital. de Biol., 1907, xlviii. 430.
(17) Cannon and Nice: This Journal, 1913, xxxii, 44.
(18) Gruber: Endocrin., 1919, iii, 145.
(19) Hoskins, Gunning and Berry: This Journal, 1916, xli, 513.
(20) Meltzer and Auer: This Journal, 1904, xxviii, 51.

Digitized by Google

SENSORY STIMULATION BY UNSATURATED ALCOHOLS, POLYHYDRIC ALCOHOLS AND CHLORHYDRINS

MARIAN IRWIN

From Radcliffe College, Cambridge, Massachusetts

Received for publication December 17, 1921

It is commonly stated that in many respects the unsaturated monohydric alcohols resemble the saturated; and that in each series of polyhydric alcohols the members act much alike, and that to a considerable extent their chlorhydrins behave like them. It is of interest to see how much correlation exists between the chemical behavior of such organic substances and their action on living protoplasm.

The efficiency of such alcohols as allyl alcohol, ethylene glycol, glycerol and of the chlorhydrins, ethylene chlorhydrin and glycerol chlorhydrin, was tested by noting their effect on the sensory cells of *Allolobophora foetida*. These results are to be compared with the effects of monohydric alcohols which were studied in a previous investigation (1).

The method used was the same as that described in a previous paper (2). When a worm is placed on a table, surrounded by a test solution, the worm will slowly crawl to the edge of the table and enter the solution. The time elapsing from the moment the prostomium enters the solution until it is withdrawn is the reaction time of the worm to the test solution.

Comparison of figure 1 with figure 2 shows that the efficiency of allyl alcohol is much greater than that of either glycol or glycerol. The behavior of allyl alcohol is more comparable with that of propyl alcohol than with that of the polyhydric alcohols. Glycol, which has one carbon atom and one OH group less than glycerol, is more efficient than glycerol, though both glycol and glycerol are very inefficient as compared to allyl alcohol.

Since high concentrations are used, the effect of glycol and glycerol might be interpreted as due entirely to the extraction of water. However, on comparing the osmotic pressures of glycol and glycerol it seems that the effect cannot be entirely due to the extraction of water, since at the concentration of these two alcohols which bring about the same reaction time, the osmotic pressures of the alcohols are different.

Digitized by Google

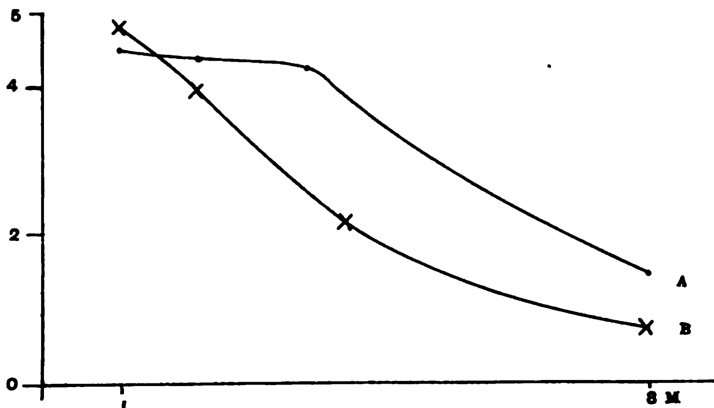


Fig. 1. The effects of ethylene glycol (curve *B*) and glycerol (curve *A*) as stimuli for worms. The reaction times in seconds are plotted as the ordinates, and the molar concentrations as abscissae.

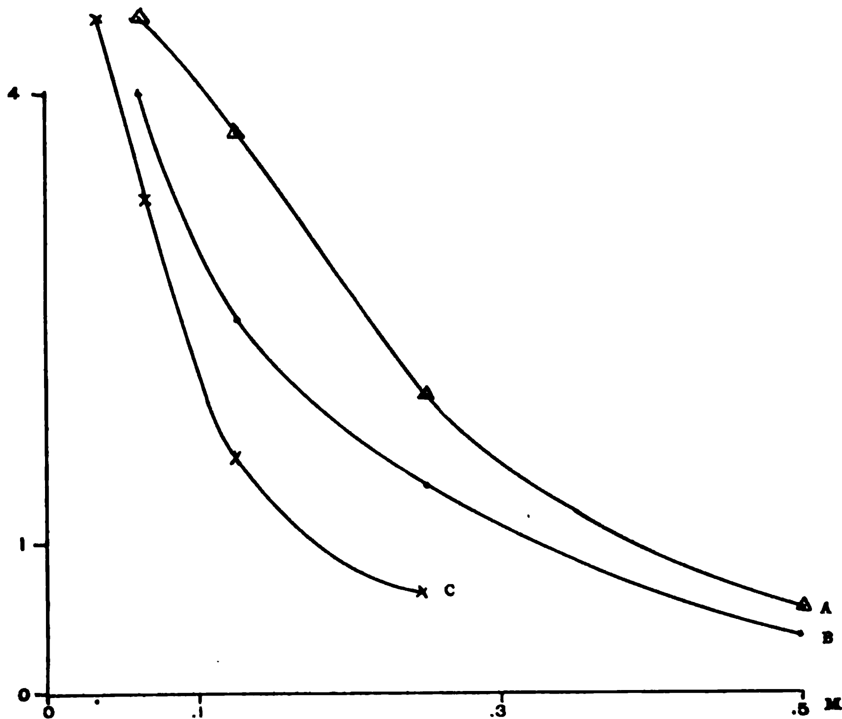


Fig. 2. Efficiency of allyl alcohol (curve *A*), ethylene chlorhydrin (curve *C*), and glycerol monochlorhydrin (curve *B*), as stimuli for worms. The reaction times in seconds are plotted as the ordinates, and the molar concentrations as abscissae.

Digitized by Google

It is commonly stated that in many respects the increase in the number of hydroxyls is of no great consequence in the ordinary chemical reactions of such alcohols, but the case is different as regards their effects on the sensory cells, since the more OH groups, the less efficient the alcohols are in producing stimulation.

As for the chlorhydrins, ethylene chlorhydrin and glycerol monochlorhydrin were used. Figure 2 shows that glycerol monochlorhydrin is slightly less efficient than ethylene chlorhydrin. Greater difference is found, however, when glycerol monochlorhydrin is compared to glycerol, and ethylene chlorhydrin is compared to ethylene glycol. Replacement of one OH group by one chlorine atom increases the efficiency several times. The H-ion concentrations of the two chlorhydrins were carefully tested; it was found that this increase in efficiency is independent of the H-ion concentration. This increase may be due to the fact that the chlorhydrins hydrolyze on entering the sensory cells. Such a hydrolysis has been described by workers on mustard gas (3).

TABLE 1

Narcotic action

SOLUTION	CONCENTRATION BELOW WHICH NO CESSATION OF MUSCULAR ACTIVITY OCCURS IN 1 HOUR AT 23°C.
Allyl alcohol	0.5 M
Ethylene chlorhydrin	0.5 M
Glycerol monochlorhydrin	0.5 M
Ethylene glycol	2 M
Glycerol	1 M

As for the narcotic effect of the given reagents, table 1 shows that allyl alcohol and chlorhydrins are more efficient than glycerol and glycol, which agrees with the stimulatory effects of the same reagents, as previously mentioned. One other distinction should be mentioned here between these two groups of reagents. The limiting concentration necessary to bring about narcosis is higher than the limiting concentration for stimulation. This is true for saturated monohydric alcohols as well as for allyl alcohol and chlorhydrins. This is to be expected, since for sensory stimulation only the prostomium, which is the most sensitive portion of the worm, is involved, while narcosis affects the entire body of the worm, which decreases in sensitivity posteriorly.

Digitized by Google

On the other hand, with polyhydric alcohols (glycol and glycerol) the limiting concentrations are not higher for narcosis than for stimulation; in the case of glycol they are the same as for stimulation and in case of glycerol they are lower than for stimulation. This distinction may be due to the fact that the effect of the former group depends primarily on chemical action while the effect of the latter group depends partly on chemical action and partly on the extraction of water. This supposition is rendered probable by the following facts. At the concentration in which the reaction time is about two seconds, there is no narcosis when saturated monohydric alcohols, allyl alcohol and chlorhydrins are used, while there is narcosis when glycol and glycerol are used. When the worm thus narcotized by glycol or glycerol is removed from the solution and placed in water, the motility of the worm is resumed very slightly for a time but this is followed in many cases by death. This inhibition of motion is perhaps due to the extraction of water from the body; although the motility of the worm is recovered to a very slight extent after the worm is replaced in water, the extraction of water has apparently gone so far that recovery is not possible. Thus it seems that the apparent narcosis due to the effects of glycol and glycerol on the worm may depend to a great extent on the extraction of water and not on chemical action.

SUMMARY

The experiments show that while the behavior of allyl alcohol toward the sensory cells of the worm is just what would be expected on the ground of its ordinary chemical relations, this is not the case with polyhydric alcohols and chlorhydrins. From a chemical standpoint, we should expect no such difference in behavior among the polyhydric alcohols themselves (and this would also apply to their chlorhydrins) as we find that in sensory stimulation where the efficiency decreases with the addition of OH groups, and a great increase in efficiency occurs when an OH group is replaced by a chlorine atom.

BIBLIOGRAPHY

(1) Irwin: This Journal, 1922, lx, 151.
(2) Irwin: This Journal, 1918, xlvii, 265.
(3) Lillie, Clowes and Chambers: Journ. Pharm. Exper. Therap., 1919, xiv, 75.

Digitized by Google

SUCCESSIVE STIMULATION BY ALCOHOLS

MARIAN IRWIN

From Radcliffe College, Cambridge, Mass.

Received for publication December 17, 1921

In a previous paper (1) it was shown that in successive exposures of *Allolobophora fœtida* to a salt solution (such as potassium chloride) an increase in sensitivity took place at certain concentrations. This was interpreted to mean that there was a definite combination of the stimulating reagent with some constituent of the sensory cell.

It is of interest to investigate the action of alcohols from this point of view. For this purpose the effects of a series of monohydric saturated alcohols, methyl alcohol, ethyl alcohol, n-butyl alcohol and iso-amyl alcohol have been studied.

Successive contacts of the prostomium of the worm with a solution of alcohol may bring about a definite change in sensitivity which depends upon the concentration. With the alcohols studied it was found that if we employ a concentration giving a reaction time of less than one second, a decrease of sensitivity takes place as the result of successive exposures. This is evident from the gradual lengthening of the reaction time, as shown in figure 1.

At a concentration lower than this, a shortening of the reaction time (increased sensitivity) is observed after a few successive exposures, as shown in figure 2. At a still lower concentration (as shown in fig. 3) there is no change in the reaction time, though the prostomium of the worm is repeatedly exposed.

We may account for these facts by supposing that the alcohol, A, unites with a substance, X, in the prostomium to form a compound AX, which is necessary for stimulation. On this basis we should expect the reaction time to shorten as the concentration of the reagent in the solution increases.

It is evident that no stimulation occurs until this compound reaches a definite concentration (threshold effect). It is also evident that this compound tends to disappear, since otherwise the weakest concentrations of alcohol would stimulate in the course of time, which is not the case.

Digitized by Google

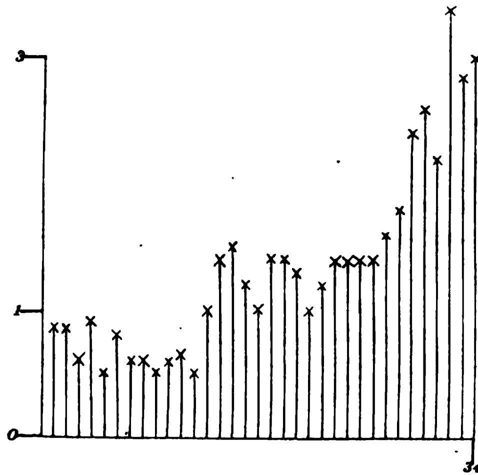


Fig. 1. Successive reactions of worms exposed to 1 M ethyl alcohol. Reaction time in seconds is plotted as ordinates, successive exposures as abscissae. This shows the lengthening of the reaction time which is characteristic of high concentrations of alcohol.

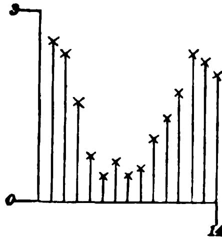


Fig. 2. Successive reactions of worms, showing an initial decrease in reaction time followed by an increase. This effect is characteristic of concentrations of alcohols somewhat lower than those which give the effect shown in figure 1. The data were obtained with 0.5 M ethyl alcohol. The reaction times in seconds are plotted as ordinates and successive exposures as abscissae.

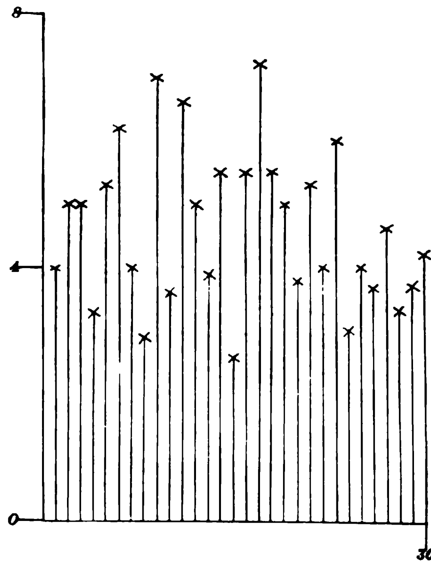


Fig. 3. Successive reactions of worms showing no change in the average reaction time. This effect is characteristic of concentrations of alcohols lower than those which give the effect shown in figure 2. The data were obtained with 0.25 M ethyl alcohol. The reaction times in seconds are plotted as ordinates and successive exposures as abscissae.

Digitized by Google

After any exposure a certain amount of alcohol remains in the tissue and this must augment the effect of the alcohol which enters at the next exposure. Hence the reaction time will shorten, unless the concentration of alcohol is so low that the compound AX disappears as fast as it is formed, so that the augmentation is ineffective. As a matter of fact we find that the reaction time does shorten in case of the above experiments as well as in case of those with the potassium salts.

The rate of formation of AX depends on the concentration of both A and X and it is therefore evident that as the substance X is used up the reaction time must lengthen.

If the concentration of alcohol is high enough X is used up so rapidly that the reaction time begins to lengthen after a few exposures have been made.

If there is a sufficiently long interval between exposures, X is more or less completely restored and a corresponding recovery of sensitivity will take place.

This hypothesis seems to afford a simple explanation of the facts without involving any unreasonable assumptions.

SUMMARY

1. A series of monohydric saturated alcohols was used for a repeated stimulation of worms at different concentrations. In the case of each alcohol it is found that there is a certain concentration at which successive exposures bring about a decrease in the sensitivity of the worm, concentrations somewhat lower than this bring about an increase in sensitivity, and concentrations still lower bring about no change in sensitivity.

A hypothesis is developed to account for these facts.

BIBLIOGRAPHY

(1) Irwin: This Journal, 1918, xlvii, 265.

Digitized by Google

THE EFFECT OF VARIOUS SALTS ON THE OUTGROWTH FROM EXPERIMENTAL AMOEBOCYTE TISSUE NEAR THE ISOELECTRIC POINT AND WITH THE ADDITION OF ACID OR ALKALI

LEO LOEB AND KENNETH C. BLANCHARD

From the Department of Comparative Pathology, Washington University Medical School, St. Louis, and the Marine Biological Laboratory, Woods Hole, Mass.

Received for publication December 19, 1921

The starting point of our investigations was observations which we made in the year 1905 and 1906 (1). At that time we observed that in isotonic NaCl solutions Limulus cells sent out pseudopodia, but soon lost their granules and became hyaline. In acid and alkali solutions the cells rapidly took up water, swelled and lost their granules; in addition there were in acids some changes which were due to coagulative processes which were lacking in alkali. Thus neutral isotonic NaCl solutions as well as acids and alkali were more or less injurious. If, however, acid or alkali and neutral isotonic NaCl solutions were combined, they mutually counteracted their injurious influence and the cells remained well preserved. Both, small amounts of acid and alkali had this effect. HCl was in this respect much more potent than acetic or citric acid in accordance with the greater electrolytic dissociation of HCl. The fact that both acid and alkali were beneficial, while at the neutral point the cell granules were dissolved, we referred tentatively to the amphoteric character of the cell proteids (1907). Subsequently Michaelis and Takahashi (2) found that hemolysis of erythrocytes under the influence of acid takes place at a pH which represents the optimum for the coagulation of the stroma particles. This corresponded to the isoelectric point of the stroma. Jodlbauer and Haffner (3) studied hemolysis by heat, narcotics and hypotonic substances and interpreted their results in a similar way. R. Hoeber and R. R. Spaeth (4) likewise referred quantitative relations which they observed in analysing the effect of certain trivalent kations on the contractility of muscle to the variation in the electric charge of the colloids, and to a greater liability to injury of the solutions at the isoelectric point.

Digitized by Google

As to the antagonism between neutral salts and acids and their mutual neutralizing effects, a considerable number of important results has been recorded. Jacques Loeb (5) first observed in 1899 that acid increased the affinity of muscle for water. Neutral solutions of NaCl also favored water absorption by muscle; but if acid was added to a hypertonic solution of NaCl, it caused a diminution in the taking up of water. Alkalies on the other hand always increased the absorption of water. The antagonism between acid and salts was in later years studied by R. S. Lillie (6) in its effect on the ciliated epithelium of Mytilus, by Jacques Loeb under varied conditions in its effect on embryonic and adult Funduli (7). W. J. V. Osterhout (8) determined variations in the electrical resistance of Laminaria under the influence of different electrolytes and with this method showed that also in the case of plants an antagonism exists between acids and neutral salts which affects primarily the permeability of the cells.

Of special interest is the observation of this author that acids decrease and alkali increases the permeability of Laminaria. These authors agree that the antagonism between neutral salts and acids depends upon the effect of these substances on the cell membranes.

The most recent studies are those of M. E. Collett (9), who found similar antagonisms in the effect of salts and acids in the case of Infusoria. Some of her conclusions very closely parallel our earlier observations on the amoebocytes of Limulus.

Our present investigations carry further our earlier work. We studied in vitro the effect of solutions of various neutral salts on the cultures of experimental amoebocyte tissue with and without the addition of variable amounts of acid and alkali. After preliminary experiments had demonstrated (10) that cells may show very active migration in such acid solutions, sometimes surpassing those in neutral solutions, we now found that through both addition of acid and alkali to neutral solutions not only an extremely marked improvement in the outgrowth of the amoebocytes may be obtained, but that the outgrowth in acid may even surpass in extent and duration the outgrowth in Limulus serum which represents the natural and otherwise most favorable medium for the migration of the amoebocytes, and furthermore that solutions of neutral salts, which are an unfavorable medium for the amoebocyte tissue, may be converted into a favorable one by the addition of acid and, although less strikingly, by the addition of alkali.

1. *Method and variable factors.* The method used in these investigations is the same as in the two previous investigations. We prepared

Digitized by Google

experimental cell fibrin (Amoebocyte) tissue of Limulus and we placed small pieces of this tissue on cover glasses. We surrounded the pieces with fluid and inverted the cover glass over a hollow glass slide, fixing it to the latter with vaseline. We made thus tissue culture preparations of experimental Amoebocyte tissue.

In our preceding publications we called attention to some variable factors which may occur in such experiments. They are as follows: *a*, The size of the piece is of importance. We used on the average pieces about 3 mm. square; if the pieces are much smaller, the outgrowth is usually less extensive. The cut surfaces ought to be straight; irregular and torn outlines at certain places may signify that the tissue has been injured and diminished outgrowth may result from this injury. *b*, It is advisable to surround the pieces with a considerable amount of fluid. We used especially deep and wide hollow slides. If the amount of fluid surrounding the pieces is small, the effect of the various solutions may become obscured. Either firm, tissue-like or soft mucoid material can be used. Cell fibrin as it is obtained about 5 to 6 hours after withdrawal of the blood is usually soft, and it has to be scraped off the dish in order to place it on the cover glass. On the following day such material is usually tissue-like and can be cut into regular pieces; but sometimes it is still mucoid even after having been kept 20 hours in the ice chest. Tissue 2 days old or older usually retracts when cut and generally is inferior to fresh tissue. In our recent experiments we found indications which pointed to the presence of an additional variable factor. In the two preceding summers (1919 and 1920) in the large majority of experiments the tissue grew well in isotonic NaCl solutions; on the second day the outgrowth in this solution not rarely surpassed that in Limulus serum, while the latter proved usually superior on the first day. In the experiments of the summer 1921 the tissue grew well in an isotonic solution of NaCl only in about 10 per cent of the experiments; in the large majority of the experiments the outgrowth in this solution was very slight or missing altogether; instead we found signs of the destruction and solution of the cells and of the margin of the piece in these salt solutions, although the experiments were carried out under sterile precautions. The difference is due either to a variability in the amount or character of substances extracted from the piece, or to variations in the resistance of the cells in different individuals. We have called attention to the possible influence of variations in the amount of serum or other substances extracted from the tissues in our previous paper. It seems most probable that the animals were less resistant in

Digitized by Google

1921. We obtained the best results in isotonic NaCl solutions in experiments in which the tissue was till loose and very sticky after having been kept in the ice chest for 20 hours or more. In such cases the cells evidently had less tendency to undergo those changes which lead to a retraction of the tissue. On the other hand, tissue which was equally loose only 5 hours after the withdrawal of the blood usually did not give better results, although it contained presumably a relatively great amount of serum. It is probable that under favorable conditions healthy cells migrating out of the tissue, aided perhaps by the presence of substances extracted from the tissue, can resist otherwise adverse environmental conditions. Thus we found in our previous experiments that even in hypertonic as well as in hypotonic NaCl solutions cells may migrate out of the pieces and that even in these solutions the character of the pseudopods may remain unaffected by the environment for some time. Only at certain periods in their history the cells show the typical changes. It is probable that the power of resistance varies in different animals.[1]

The effect of Limulus serum is much more constant. We must therefore in the first place compare experiments made with the same tissue, and only secondarily can we compare with each other the results obtained with different tissues.

The effect of Limulus serum. As in our previous experiments, Limulus serum proved to be the best medium in the first 24 hours. Later extension and gradual hyalinization of the cells occurred and this made an end to the further outgrowth. Limulus serum gave the most constant results; they were less affected by variations in the character of the tissue. Some variations however occurred even here; tissue which was still mucoid on the second day gave not only with isotonic NaCl solution but even with Limulus serum especially favorable results. This indicates that the beneficial results obtained with this tissue were in all probability not due to admixed serum. In Limulus serum the typical cell clumps formed and all varieties of tongue pseudopodia could be observed.

Even Limulus serum that had been kept by us for a period of about 11 months in test tubes, sealed with paraffin, and that had previously

[1] The winter and spring of 1920 and 1921 were unusually mild and it is possible that this climatic condition is responsible for the difference noted. It remains, however, for future investigations to determine which of the factors mentioned is responsible for this variability. It will also be necessary to consider the possibility that in some cases the blood was infected in the living animal.

Digitized by Google

been heated to 54° for half an hour, was found to be just as effective as fresh serum. As we stated previously, sera of other arthropods cannot take the place of Limulus serum. The effect of Limulus serum is therefore specific.

The effect of isotonic solutions (m/2 and 5/8 m NaCl). As stated above, in the large majority of the experiments, no or very little outgrowth was obtained in these solutions. Those cells that did grow out soon spread out and became hyaline and cytolysed. As a result of solution processes in the migrating cells or in the margin of the piece a finely granular precipitate often formed around the piece, or sometimes the viscous cell material was drawn out into radiating fibers which surrounded the margin of the piece. In other cases a layer of granular cells which were suspended in the fluid surrounded the piece. In several cases a slight outgrowth of granular contracted cells occurred. Gradually these cells spread out and became hyaline. In five experiments, however, a very good outgrowth was obtained similar to that recorded in previous years. In some of these cases it exceeded even the growth in Limulus serum especially on the second day.

These cells showed the characteristics formerly described. There was less agglutination in a m/2 NaCl solution than in Limulus serum. In the former the cells were on the whole more evenly distributed, although clumping did occur. The cells were more contracted and had somewhat sharper pseudopodia than in Limulus serum. Many cells showed in their posterior half furrows due to a localized contraction of the protoplasm. The cells being less sticky, a number of them detached themselves from the surface of the cover glass and fell down and formed a second layer of cells on the lower surface of the drop. This layer was usually smaller than the layer at the surface of the glass.

In the majority of our recent experiments the effect of an isotonic NaCl solution on the amoebocytes is in accordance with the result of those of our former experiments in which we received a drop of blood directly into a NaCl solution, or in which we poured an isotonic NaCl solution on the amoebocytes. Under those conditions we found the cells to send out rather sharp pseudopods and very soon afterwards to lose their granules and to become hyaline and cytolysed.

In order to explain the outgrowth of the cells in isotonic NaCl solutions, we had to assume that many cells growing out from the experimental amoebocyte tissue were protected in some way against the injurious effect of the NaCl solution, at least for some time. Later, however, they also degenerated. We considered previously the possi-

Digitized by Google

bility that substances might be extracted from the piece and that these protected the cells.

The effect of the addition of acid and alkali to the isotonic NaCl solution. The addition of acid to the isotonic (5/8 m to m/2) solution of NaCl caused in almost all experiments a marked improvement. One part of HCl solution varying in strength between m/10 and m/1000 was added to ten parts of the NaCl solution. The strongest concentrations caused a solution of the margin of the piece. The optimal action was obtained with m/100 to m/200 HCl. In the majority of cases m/100 HCl represented the optimal quantity; but in some experiments m/80 HCl likewise produced an improvement. In some cases m/200 lactic acid, m/200 HCl, or m/200 to m/400 butyric acid were optimal.

In the case of the organic as well as of the inorganic acids the optimum varied slightly in different cases, and in one case the optimal effect was found after addition of m/300 to m/400 acid (in the proportion of 1 part acid to ten parts salt solution).

Weaker solutions up to the strength of m/400 HCl still were advantageous; but usually the effect of weaker solutions was less marked than that caused by the optimal solutions. Still weaker solutions usually were without effect. In the fluid surrounding the piece the optimal concentration of acid corresponded therefore to somewhat less than m/1000 to m/2000 HCl solution; but a further deduction has to be made on account of a slight admixture of serum which was invariably adherent to the piece. It caused a precipitate with the acid and this precipitate surrounded the piece usually leaving a clear area directly around it. The proteid as well as inorganic buffer solutions contained in the admixed serum and dissolved parts of the tissue reduced, of course, the amount of free acid present in the fluid.

Not only HCl had this beneficial effect, but also organic acids, of which butyric and lactic acid were used. Although the electrolytic dissociation of the organic acids used is considerably below that of HCl, still their effect was very similar to that of HCl and they could be used in similar concentrations. Again addition of 10 per cent of a m/100 to m/200 solution of butyric acid and lactic acid was found best. We must therefore assume that acid does not altogether act through the changes in hydrogen ion concentration which it produces; but that the anion or the undissociated acid may play a certain part.

In a few cases no effect of the acid was obtained. These were cases in which only one concentration of the acid was tested and in which the observation extended only over the first 24 hours. In a complete experi-

Digitized by Google

ment a series of dilutions ought to be used and the observation ought to extend over several days. If that is done, positive results ought to be obtained in every case, except when the cells are already much deteriorated in the beginning of the experiment.

The results of addition of acid are most striking, when tissue is used which shows only a trace of growth or no growth at all in a neutral NaCl solution; then we find in many cases an excellent outgrowth in the acid solution which is often better than that in Limulus serum, especially on the second or third day; but even in those cases in which already in the neutral NaCl solution the outgrowth is very good, the acid produces improvement. This may be noticeable as early as 24 hours after the beginning of the experiment, but it becomes more marked after 2 or 3 days or even later.

At that time very decided retrogressive changes have taken place in the neutral solution, while in the acid the cells may still be very well preserved and active; new cells continue to move from the tissue while the cells which had already emigrated from the tissue extend still further into the surrounding fluid.

Through varying the strength of the acid we can produce graded effects on the tissue. If the amount of the acid added is very great (10 per cent of m/10 to m/40 HCl) solution processes take place in the tissue and in the cells; a few typical acid cells may be found. If the acid added is somewhat weaker (m/80 to m/100 HCl), the cells may at first appear as fixed oval or round granular cells with sharp margins. There may be a few sharp threadlike pseudopods or pseudopods may be absent altogether. Sometimes the effect of this solution is so strong that the cells remain in this fixed condition, but in other cases a slight movement of the amoebocytes out of the piece continues slowly, but steadily, and it may proceed for a considerable number of days We could follow it for 6 days; but there is very little doubt that under favorable conditions it may continue longer. The cells under those conditions are relatively contracted, show on the whole sharp pseudopodia and furrows in their posterior part. The granuloplasm is tight and moves connectedly and slowly into the pseudopods. If the optimal concentration is reached the growth is very extensive and active already on the first day. The character of the cells is on the whole the same; the cells are contracted, granular; their extension and hyalinization is very slight. On the first day addition of 10 per cent of a m/200 HCl may sometimes be as good as of a m/100 HCl solution; but on the second day the weaker solution usually allows a greater extension and subsequent hyalinization of the

Digitized by Google

cells. The weaker the acid is, the more it allows in the course of time extension and the degenerative processes which follow it to take place, although at first the acid may still exert a beneficial effect. A stronger solution usually shows more inhibition on the first day but it continues to keep the cells in a contracted and actively moving state for a longer period of time. It prevents very much more efficiently the spreading out of the cells and its injurious consequences.

In weaker acids the result is therefore more and more like that in neutral NaCl solutions; at first this weakening of the effect appears only after the lapse of some time; but if still weaker acid is used, it becomes apparent at once. The stronger solutions keep the cells contracted and, if used in optimal quantities, still sufficiently soft to permit active movement; if the acid action is still greater the cells become so hardened and fixed that, while extension is prevented, at the same time movement is also completely inhibited.

In accordance with this effect of acid on the consistency of the cells or its outer layer the pseudopods are generally made sharper through the addition of acid; occasionally, however, some drops are observed even in acid solutions, especially in the most advanced cells, which are exposed to the full effect of the solution.

Organic acids act on the whole like HCl; but in some cases it appeared as if possibly the cells were somewhat softer in the solutions of organic acids and some cells showed perhaps some broader pseudopods than in solution of HCl.

While thus acid in suitable concentration tends to preserve the blood cells, to prevent the spreading out of the amoebocytes and the retrogressive changes which follow this extension, this effect is after all not of indefinite duration, but after some time the cells will extend and hyalinize and become destroyed even in an acid solution.

The rapidity with which these secondary events occur depends upon the strength of the acid. If it is so strong that the migration is slow and the cells relatively little motile, as if it were hardened, the extension and subsequent retrogressive changes take place less rapidly; in weaker acid, as after addition of m/200 HCl, the extension and hyalinization usually occur more rapidly and may be noticeable already on the second day. Some cells, of course, in direct contact with the cover glass and situated in the periphery of cell clumps may extend and hyalinize as early as on the first day. But even extended cells may preserve their granules longer in acid than in a neutral solution.

Digitized by Google

The rapidity and intensity of outgrowth in an acid solution may be so great that it may surpass already on the first day the zone of growth in Limulus serum. It usually exceeds the growth in Limulus serum on the second day, when in the latter the complete extension and subsequent retrogressive changes preclude any further progress.

The effect of alkali. One part of a m/10 to m/20 NaOH solution added to 20 parts of m/2 or 5/8 m NaCl have also often a beneficial effect on the preservation of the cells and on the outgrowth from the tissue, although the effect is usually not so marked as in optimal solutions of acid, but it may approach this effect. Addition in the same proportion of solutions much stonger than m/10 NaOH has a dissolving effect on the tissue, and solutions much weaker than m/20 NaOH are usually without decided effect, although perhaps there may be a slight temporary influence noticeable from solutions as weak as m/30 or m/50 NaOH. Again as in the case of the acid the amount of alkali present in the solution is diminished through the admixture of some serum or dissolved cell material to the fluid.

Alkali counteracts the dissolving effect of the neutral salt solution and thus preserves the cells. The character of the migrating cells in alkali is similar to that in acid solution; the cells are contracted; a large number of the pseudopods are sharp tongue pseudopods. There exist however some distinct differences between the action of acid and alkali. Alkali usually permits a much larger number of cells to extend and to become dissolved at an early period than acid does. In consequence the field of emigrated cells is in alkali usually thin, while in acid it is densely packed with cells, the more so the nearer the area of growth is to the piece of tissue. In alkali many cells become dissolved, and this applies not only to extended cells, but sometimes even to cells which have not yet fully spread out, and thus there may be found considerable interstices between neighboring cells. These are now free from cells, but cells had been here previous to their solution.

On the second day retrogression has usually become much more marked in alkali than in acid. This combination of the transient preserving effect of alkali added to its tendency to permit after some time the spreading out of well preserved granular cells, may make the picture presented in an alkaline solution not unlike that seen in Limulus serum, where also the cells generally extend in a granular form, the granules remaining preserved for some time following extension. The cells are therefore gradually softening in alkali and thus it comes about that while sharp tongue pseudopodia are quite common in alkaline solutions, broad

Digitized by Google

and round pseudopods seem to be more frequent here than in an acid solution.

The effect of acid and alkali in hypertonic NaCl solution (3/4 m NaCl solutions were used in these experiments). As in the case of the isotonic NaCl solutions, we found here again variability in the results. In many cases the emigrating or marginal cells hyalinized and cytolysed rapidly and the solution of the tissue led to the formation of an opaque zone and of granular material around the piece. In other cases a zone of contracted granular cells formed around the tissue. In accordance with our former observations the pseudopods were usually found sharp, often threadlike, although other kinds of tongue pseudopodia were also found quite commonly, and sometimes even small drops developed. The average of the pseudopods was however quite distinctly sharper here than in isotonic and hypotonic solutions. Gradually the NaCl solution penetrated into these cells and made them hyaline, especially after they had spread out; but even in contracted cells the hypertonic NaCl solution could enter and cause a solution of the granules. Temporarily the hypertonic solutions preserved the cells in some cases better than isotonic solutions; but whenever a good outgrowth was obtained in NaCl solutions, the zone of outgrowth was greater in isotonic than in hypertonic solutions.

Addition of acid in the same proportion as in the case of isotonic solutions proved in a number of instances beneficial. The area of outgrowth was enlarged and the time during which the outgrowth persisted was increased. In one case we followed the progress of outgrowth over a period of 5 days during which the cells were mostly kept at room temperature. During this whole period the majority of the cells was well preserved, contracted granular, and the pseudopods were on the average very sharp. The extreme sharpness of many pseudopods is characteristic of a combination of hypertonicity and acid reaction. The granuloplasm moves connectedly into the pseudopods in accordance with the great consistency of the protoplasm under these conditions. We find here the sharpest, most threadlike pseudopods, provided the solution permits an emigration of the cells. We find here the same graduation in the strength of the effect of acid as we did in the case of isotonic solutions: we observe all transitions from solutions which fix the cells and thus prevent an outgrowth to optimal solutions in which the consistency of the protoplasm is increased but outgrowth is possible, and to ineffective ones.

Digitized by Google

Again the optimal solution is obtained through addition of about 10 per cent of a m/100 to m/200 HCl solution. In some cases m/100 HCl was optimal, in others m/200 HCl. In some cases it appeared as if in the case of hypertonic solutions the optimum was at a somewhat greater dilution (m/200 HCl) than in the case of isotonic solutions; but if this should prove to have been more than a coincidence, it cannot apply to all cases, because in some instances m/100 HCl was more favorable than m/200 HCl. It is very probable that with a proper dilution of acid hypertonic solutions will prove to be even more suitable for the long-continued emigration of cell from the piece than isotonic solutions, because in acid hypertonic solution a summation of hypertonicity and increased acidity occurs. Both tend to prevent the taking up of water into the cell, and thus prevent a lowering of the consistency of the cell. But even in hypertonic acid solutions an extension and destruction of cells sets in ultimately. Further experiments will have to decide how long this can be delayed through the selection of suitable concentrations.

In a number of cases no benefit was derived from the use of acid in these solutions. Whether in these cases the hypertonic NaCl solution in itself increased the consistency to such an extent that acid had no chance to act, or whether the proper concentration of the acid had not been found in these cases, cannot be stated at present.

In general even an optimal addition of HCl to a hypertonic solution cannot produce an effect equal to that obtained by the addition of acid to an isotonic solution. But inasmuch as in a hypertonic solution the outgrowth may in certain cases continue over a longer period the outgrowth may come to equal that obtained in isotonic acid solutions at a time when in the isotonic solution the cells had begun to extend and thus to undergo solution processes.

Addition of alkali to hypertonic solutions also produced an improvement in a number of cases; addition of about 10 per cent of a m/10 NaOH solution proved usually better than addition of a m/20 NaOH solution. But again in alkali hyalinization and extension occurred usually earlier than in acid; often on the second day or occasionally even as early as the first day. In alkali drop formation may occur even in hypertonic solutions and in one case circus movements of the drop were observed; furthermore structures resembling eggs with fertilization membranes were found. On the third day the cells in alkaline hypertonic solutions did no longer show active movement.

The effect of acid and alkali in hypotonic NaCl solutions. In m/4 and m/6 solutions of NaCl we found in the majority of experiments instead

Digitized by Google

of outgrowth an opaque or granular material around the piece, the result of a solution of the margin of the tissue. In some cases, however, an outgrowth occurred such as we obtained in hypotonic NaCl solution quite frequently in previous years. Even in the absence of a definite outgrowth we not rarely found a limited number of cells migrating from the piece; occasionally these cells were suspended in the surrounding fluid. Again we observed the occurrence of the broad tongue and balloon pseudopodia and of transitional forms in hypotonic solutions, in addition to the ordinary tongue pseudopodia which latter were quite numerous. There is evidently a gradation in the character of pseudopods in hypotonic solutions. As long as the cells resist the action of the hypotonic medium, the pseudopods and movements of the granuloplasm may be approximately normal; but when they begin to take up water and thus to diminish their consistency their pseudopods assume the form characteristic of hypotonic solutions and of a lowered consistency. Occasionally even circus movement may be observed in an isolated cell. When the effect of the hypotonic solutions becomes still more pronounced, those structures are produced which we designated as courts and others which we compared with fertilization membranes; still other cells may merely show a swollen appearance and coarse granulations. In such cells the movements of the granuloplasm have usually ceased and the cells are irreversibly injured.

In those cases in which in neutral NaCl solutions a good outgrowth was obtained, it was inferior in hypotonic solutions to that observed in isotonic solutions.

The addition of acid or alkali to the hypotonic solutions produced a distinct and often very marked improvement in many cases, but not in all. It seems that in some cases the cells are not able to resist the effect of the hypotonic solution notwithstanding the counteracting influence of acid or alkali. If we reduce in such cases somewhat the degree of hypotonicity, the acid or alkali may be rendered efficient. For instance, in cases in which a m/4 NaCl was found to be too hypotonic, addition of acid or alkali to a m/3 NaCl solution caused a great improvement in tissues with a low degree of resistance. But in many cases the typical effects of acid and alkali can be produced not only with a m/4 NaCl, but even with a m/6 NaCl solution. Addition of acid rather than of alkali may produce in such cases a remarkable improvement. And yet the extent of outgrowth in an hypotonic acid solution is usually distinctly inferior to that in an acid isotonic solution, but occasionally it may approach it, and in one case even surpassed the growth in isotonic acid

Digitized by Google

solution on the second day. The distinction due to the osmotic differences are therefore not obliterated by acid or alkali; the effects of the latter are rather added to the osmotic effects. In cases in which there was a good outgrowth in neutral isotonic NaCl solution, the addition of acid and alkali improved markedly the growth in hypotonic solutions and in other experiments in which there was no or only a very slight outgrowth in neutral NaCl solutions, acid or alkali often called forth a very good outgrowth. It is however probable that the number of cases in which addition of acid or alkali was unable to improve noticeably hypotonic solution is greater than the number of experiments in which they were ineffecitve if added to isotonic solutions.

The effect of acid and alkali, when added to hypotonic solutions, in principle is similar to that which we described in the case of isotonic solutions. Especially acid prevents or retards the entrance of water into the cell. It tends to preserve it and to prevent the solution of its granules. Again the cells in the acid solutions are contracted, frequently show the furrow in the posterior end of the cytoplasm; the cells move actively. The pseudopodia tend to be sharp and the more so, the more contracted the cell as a whole is. However, the acid and the hypotonicity of the solution tend to counteract each other, and the acid is not altogether able to prevent a decrease in consistency, and in such cases the pseudopods become broader and balloon-like; occasionally even circus movement may be observed.

While alkali resembles acid in that it tends to preserve the cells and to increase their outgrowth, it is much less effective than acid. As in isotonic media alkali tends to produce a solution of the cells to a much greater extent than the acid. It promotes the extension and subsequent disintegration of cells; but even contracted cells may be dissolved in very hypotonic solutions under the influence of alkali. Thus we are liable to find the fields of outgrowth in hypotonic alkaline solution to be very thin, attenuated. The cells not rarely have the appearance of being fixed, oval or round and flat or somewhat extended.

The combination of marked hypotonicity and alkalinity may in some cases produce changes in cells which are similar to those found in m/4 KCl solutions. Balloons, which may be multiple, and by means of which the cells may move can develop; and in some of these cells circus movements begin to take place. In such cases alkali probably penetrated into the cell in the hypotonic medium and decreased the consistency of the granuloplasm sufficiently to call forth the circus movements. The optimal quantity of acid and alkali in the hypotonic

Digitized by Google

solutions is not noticeably different from the optimum found for the isotonic solutions; it varies between m/80 and m/200 HCl and m/10 to m/20 NaOH. Lactic and butyric acids also were effective. In some cases it appeared that the stronger acid and the weaker alkali were preferable in hypotonic solutions; but in other cases no such distinction was possible.

The effect of acid and alkali in combination with m/2 and m/4 solutions of LiCl. To judge from the results of a small number of experiments the effect of LiCl in m/2 and m/4 solutions is similar to that of corresponding NaCl solutions. The outgrowth was slight, tongue pseudopodia or sometimes irregular balloons were produced; the cells underwent an early hyalinization. Addition of acid caused an improvement in a number of cases; it increased the amount of outgrowth and caused a greater contraction of the cells similar to that found in NaCl solutions. In several cases m/100 HCl caused too strong an acid effect while m/200 HCl acted favorably; in other cases however m/100 HCl was better. Acid caused an improvement even in hypotonic solutions of m/4 LiCl.

In isotonic solutions alkali preserved the cells for some time and the cells were softer, more regular than in acid solutions; they were either flat or extended and granular; the outgrowth was usually less than in acid, but in a number of cases better than in controls. In isotonic solution m/10 NaOH was optimal, while in hypotonic solutions m/20 NaOH proved to be better.

The effect of the addition of acid and alkali to isotonic (5/8 and 1/2 m) solution of KCl. In many experiments no outgrowth occurred in isotonic solutions of KCl, but instead precipitate or opacity around the piece indicated a solution of the margin of the piece. In other cases a number of cells were freed from the piece as the result of these solution processes. The cells were usually slightly swollen, round, coarsely granular or hyalin; some cells showed courts or fertilization membranes or balloons. Occasionally a small zone of outgrowth occurred; among those cells some had tongue pseudopodia or transitions between tongue pseudopodia and balloons; others showed the typical KCl character.

The movement of granuloplasm in isotonic KCl solutions was usually slight and ceased altogether after some time. The outgrowth in m/2 KCl solutions was relatively best in those experiments in which also in NaCl solutions the cells were found most resistant.

In a number of experiments addition of alkali or hydrochloric, butyric or lactic acid did not produce a distinct improvement. The small number of cells which sometimes were visible around the piece were

Digitized by Google

coarsely granular; some had courts, others were fixed and did not show any movement; in some cases it seemed that the acid increased the consistency of the protoplasm. In a number of cases acid produced some improvement, which however was marked only in a few instances.

In a case in which some improvement was produced, m/100, m/200 HCl and m/100 butyric acid were about equally beneficial on the first day; on the second day m/200 HCl proved to be better than m/100 HCl. In this case the cells became more contracted under the influence of the acid, the tongue pseudopods increased in number and sharpness; yet despite this improvement there was always some effect of potassium noticeable. Even in the cells in which tongue pseudopodia through which movement took place, had been produced, the protoplasm of the cells was more rigid, rounded off and granular, and instead of the granuloplasm secondarily moving into the pseudopods, the pseudopods were kept in an extended state and pulled the cell as a whole along. Thus the movements were not those of normal amoebocytes, but comparable to the movements of a snail which through its foot pulls the rest of the animal along. Even in specimens in which an improvement was produced through the acid, the potassium effect was still noticeable in the formation of balloon and drop pseudopodia, and even in the most favorable specimens the movements of the granuloplasm were restricted. Occasionally even circus movements occurred in acid KCl solution. There was no or only a very slight progress on the second day. Usually the potassium proved too toxic at that time and the cells were fixed and swollen. Addition of alkali rather emphasized than attenuated the potassium effects and it was therefore not beneficial; acid on the other hand counteracted to some extent the tendency of the cells in K salts to take up water; and thus in acid potassium solutions the cells were usually isolated and rarely formed clumps as they did in alkali.

The effect of addition of acid and alkali to hypotonic (m/3 and m/4) solutions of KCl. In m/4 solutions of KCl a part of the margin of the tissue was often dissolved, and precipitate or an opaque area surrounded the piece. Quite frequently we found a number of isolated cells around the piece and these showed changes characteristic of m/4 KCl solutions. The cells took up water and they became coarsely granular, balloons and typical circus movements developed. A number of cells disintegrated and their disintegration caused the formation of a coarsely granular precipitate. Especially in those experiments in which good results were obtained with NaCl solutions, the effect in the m/4 KCl solutions was likewise better; the number of cells showing circus movements on

Digitized by Google

the whole being greater, and occasionally more contracted cells with the typical furrows were observed.

Addition of alkali (1 part of m/10 to m/20 NaOH to 10 parts of a m/4 KCl solution) apparently accentuated the potassium effect; the balloons and circus movements seemed to become more frequent; otherwise no marked change was produced. Addition of acid in the usual proportions on the other hand had a tendency to decrease or to prevent the circus movements and balloon formation; although both did occur in acid solution. Frequently the cells were round, hyaline or granular, but in a number of cases tongue pseudopodia or transitions between tongue pseudopodia and balloons were formed, and in accordance with the increase in the number of tongue pseudopodia the number of outgrowing cells was distinctly increased. Again we found that those tissues which also in other solutions showed the greatest activity and resistance gave the best results after the addition of acid. On the whole acid tended to make the cells more consistent, to decrease the taking up of water and the fluidity of the granuloplasm, and thus to counteract the effect of potassium.

When the action of acid was too strong the cells were fixed; if the action was slightly less, the consistency was sufficiently great to permit the formation of tongue pseudopodia, and at the same time it left a few cells sufficiently soft to make possible the circus movements and the formation of balloons.[2]

The effect of isotonic and hypotonic solutions of RbCl and CsCl. In m/2 solutions of RbCl and CsCl the effect is intermediate between that caused by m/2 NaCl and m/2 KCl. The cells emigrate as contracted granular cells with sharp to broad tongue pseudopodia, spread out and become hyaline. In so far they resemble cells in NaCl solution; but at the same time there are noticeable some effects that are similar to those of potassium.

A number of cells are flat and coarsely granular and balloons are noticeable in addition to the tongue pseudopodia. In cases in which the outgrowth is very good in NaCl solutions, the outgrowth in RbCl and CsCl may approach that in NaCl; in such cases the cells are similar to those seen in NaCl solution; they are granular contracted and form tongue pseudopodia. When the outgrowth is very slight or lacking in m/2 NaCl solution, the outgrowth is likewise diminished in RbCl and CsCl, but it is here somewhat better than in NaCl solutions.

[2] In order to make certain that the effect of potassium, which we had described previously, was not due to an admixture, we used a KCl salt prepared by Doctor Doisy which had been specially purified and which he kindly put at our disposal.

Digitized by Google

In m/4 RbCl and m/4 CsCl the outgrowth was always better than in m/4 KCl and accordingly tongue pseudopodia were somewhat more numerous; and in this respect these solutions resembled m/4 NaCl solutions. But at the same time the formation of balloons and transitions between balloons and tongue pseudopodia was very pronounced and we found distinct circus movement in a number of cases. There were polarized and irregular balloons through which the cells could move. Also coarsely granular, flat cells were found. The greater frequency of balloons and the occasional occurrence of circus movements were effects similar to those observed in m/4 KCl. In m/4 KCl on the other hand the circus movements were more frequent and the outgrowth more limited. In these solutions we have the combined effect of hypotonicity and of specific ion effects on pseudopodia.

RbCl and CsCl are therefore intermediate between KCl and NaCl. On the whole RbCl approaches perhaps KCl more than CsCl, although the difference between RbCl and CsCl is slight.

The effect of acid and alkali in combination with RbCl and CsCl. M/100 and m/200 HCl added to isotonic solutions of CsCl and RbCl in the proportion of 1 to 10 produced a marked improvement. The outgrowth increased and the cells became more contracted, their pseudopods were sharper; but even in these mixtures the effect of Rb and Cs was noticeable and occasionally some balloons and even restricted circus movements ocurred notwithstanding the presence of the acid. RbCl and CsCl counteract therefore partially the effect of the acid. M/10 and m/20 NaOH cause an increased rarefication of the field through solution of cells, a softening of the cells, hypotonic pseudopodia and circus movements. There was thus no improvement noticeable under the influence of alkali.

In hypotonic (m/4) CsCl and RbCl acid added in the usual proportion generally causes an increase in the consistency of the cells; they are made tighter, their pseudopods become sharper. In some cases m/100 HCl was too strong in its action and did not permit outgrowth to take place; in other cases, however, it increased outgrowth. m/200 HCl had a beneficial effect in a number of cases; at the same time it permitted circus movements in a few cases. While generally acid restricts circus movements very much, it does not actually prevent them; and especially in weaker acid they may occur. The Rb and Cs effect is furthermore evident in the formation of balloons even in an acid medium. Alkali again has a softening influence and may promote the development of circus movements; it does not increase the outgrowth.

Digitized by Google

In a m/2 solution of NH_4Cl the outgrowth was slight; it was still more diminished in a m/4 NH_4Cl solution. The cells in these solutions were usually round or oval, often flat; the granules were well preserved. In extended granular cells a honeycombed appearance was noticeable, perhaps the result of the solution of some granules. Instead of well-formed tongue pseudopodia we find in NH_4Cl solutions short rudimentary pseudopods, which usually are somewhat rounded and often appear as small drops and balloons. Especially common were multiple small drops, which produced the appearance of irregular mulberry cells, not unlike those produced through exposure of the cells to a higher temperature. Some of these cells disintegrated into masses of cell granules; especially in hypotonic solutions. But even in isotonic solutions a number of cells disintegrated and became dissolved. On the whole, however, NH_4Cl had a tendency to preserve the cells and the piece relatively well at least for some time.

Addition of m/100 or m/200 HCl to the NH_4Cl solutions in the usual proportions does not noticeably increase the outgrowth, but it has some of the other typical acid effects. It tends to make the cells more contracted, to produce furrows through contraction of the protoplasm and to increase the number of tongue pseudopodia; yet it cannot overcome the effects of NH_4Cl. Multiple droplets and mulberry cells still appear. In hypotonic NH_4Cl solutions we find flat, round or oval granular cells even in an acid medium.

With alkali (m/10 and m/20 NaOH) no marked effect is produced; the typical mulberry cells are observed, but in some cases the number of tongue pseudopods and the contraction of the cells seem to have increased. Gradually cells extend in these solutions and the extended cells become dissolved.

In solutions of m/2 and m/3 $CaCl_2$ or in solutions slightly less concentrated than m/3 $CaCl_2$ some outgrowth from the piece usually takes place, but the field of growth is generally not dense and often thin. This is due to the fact that in $CaCl_2$ solution the cells readily become hyaline and are soon dissolved; this applies not only to the extended or partly extended cells, but even the contracted cells tend to undergo this change. $CaCl_2$ has furthermore the tendency to keep many cells in a contracted state with sharp pseudopodia. Occasionally the pseudopods are long and rather broad and the cell body small, round, contracted; but some normal, contracted and motile furrow cells were usually observed. In m/3 $CaCl_2$ solution we also found courts and the cells resembling ova with fertilization membranes. On the second day no

Digitized by Google

progress was usually noted; instead the cell movements had generally ceased.

In calcium chloride the destruction of cells is therefore rather prominent and the viscous material resulting from this process often assumes the form of long radiating fibers which are attached to the piece and extend into the surrounding fluid.

Addition of HCl (1 part m/50 to m/200 HCl to 10 parts $CaCl_2$ solution) does not cause any decided change. The character and fate of the cells is not definitely altered. Sometimes the effect of $CaCl_2$ seems to be accentuated through the addition of acid; at other times it increases perhaps slightly the number of healthy contracted furrow cells. Addition of alkali in the usual proportions does not produce any notable changes.

In solutions of $MgCl_2$ the outgrowth of the tissue varies according to the concentration used. In a 2/9 m $MgCl_2$ solution, the margin of the tissue is dissolved, precipitate is found around the piece and no growth takes place. In a m/3 $MgCl_2$ solution some cells suspended in the fluid are found around the piece. These cells send out tongue pseudopodia and become hyaline after some time. As a result of cytolysis radiating fibers around the piece are produced. In a 5/12 m $MgCl_2$ solution the result is similar, the outgrowth is slight. Contracted granular cells with sharp pseudopodia or flat irregular cells become hyaline at an early period. In a m/2 $MgCl_2$ solution the outgrowth was larger, the cells were better preserved and clumps of granular contracted cells broke away from the piece and moved into the fluid. Subsequently many cells extended and became hyaline; and gradually even granular contracted cells became hyaline. While in the m/2 solution the outgrowth was better than in the other $MgCl_2$ solutions, the cells became hyaline more rapidly than in Limulus serum.

Addition of m/50 or m/200 HCl in the usual proportions to solutions of $MgCl_2$ did at best produce only a slight improvement in the outgrowth. m/80 or m/100 added to m/2 $MgCl_2$ increased perhaps the number of contracted furrow cells with sharp pseudopods; in other cases however the effect of this solution was too strong and merely very contracted granular cells were visible. In 2/9 m $MgCl_2$ solution to which acid had been added some round, granular or spindle shaped cells with tongue or thread pseudopodia were found around the piece. They had probably become detached from the piece through solution processes. No decided improvement was produced through acid in this case.

Digitized by Google

Solutions of m/2 and 5/8 m $NaNO_3$ behaved somewhat similar to isotonic solutions of NaCl, but in a number of cases the outgrowth was slightly better in the former. The cells were somewhat better preserved in the $NaNO_3$ solutions in experiments in which a NaCl solution proved to be an unfavorable medium. As usual the cells emigrated from the piece as granular contracted cells, they became flat and extended and in this condition the hyalinization or solution of the cells occurred. $NaNO_3$ seemed to make the cells softer and thus to favor their extension.

Addition of m/100 to m/200 HCl in the usual proportion caused in some cases a better preservation of the cells and increased the outgrowth in isotonic solutions of $NaNO_3$; the cells were more contracted and extension and hyalinization delayed; but even under favorable conditions some solution of cells occurred even in acid media in $NaNO_3$ solution. In other cases the acid was either too strong, and led to a hardening and fixation of the cells, or it was too weak and not able to overcome the softening and dissolving effect of $NaNO_3$. m/400 HCl was without effect. It seems that acid is not quite so effective with isotonic $NaNO_3$ solutions as with NaCl solutions. However, it is possible that some accidental variations may be responsible for this difference. A distinct improvement, however, was usually produced in m/2 $NaNO_3$ solutions, through addition of alkali (m/10 or m/20 NaOH). The cells were better preserved, hyalinization delayed and the outgrowth increased; but also in alkali, solution and hyalinization occurred occasionally even in contracted cells.

In m/4 $NaNO_3$ some outgrowth took place, but it was usually slight and the emigrated cells hyalinized after some time. Addition of m/100 to m/200 HCl in the usual proportions produced a very marked improvement. The cells became contracted, the pseudopodia very sharp, the consistency of the protoplasm increased and extension was much delayed. If the solution had a very strong effect and called forth a marked contraction of the cells, the beneficial effect became sometimes clear only on the second day. In alkali some improvement was also obtained, but in the alkaline solutions there was more extension and solution of the cells.

In an hypertonic (3/4 m) solution of $NaNO_3$ the outgrowth was in a number of cases better than in 3/4 m NaCl solution; the cells in hypertonic $NaNO_3$ solution seemed to be somewhat softer than in 3/4 m NaCl. Usually extension and hyalinization and cytolysis followed in this as in the isotonic solution; and in some cases the margin of the piece was dissolved and formed a precipitate.

Digitized by Google

Addition of m/100 and m/200 HCl led to an improvement which in some cases was very marked; but after some time hyalinization occurred even here. In alkali likewise improvement in outgrowth and preservation took place; but here the process of solution of extended, and even of not extended cells, was more marked than in the case of acid.

The effect of m/2, m/3 and m/4 Na_2SO_4 solutions on the outgrowth and character of cells. In solution of m/2 Na_2SO_4 we usually find a small zone of cells surrounding the piece. The cells are very contracted, round and at first granular; the pseudopods are sharp, often assuming the character of multiple threads. In accordance with this marked contraction and the sharpness of the pseudopods the cells are found to be little sticky and not to agglutinate very readily. At an early period many of these contracted cells become hyaline.

Addition of m/100 to m/400 HCl does not change very markedly the character of the cells; they remain contracted and round and with sharp pseudopods; but the granules are preserved for a longer period of time. The number of cells which surround the piece is much increased; they usually are arranged in two layers, one at the top and the other at the bottom of the drop. The cells are isolated. In a weaker (m/300) (1:10) HCl solution furrow cells of a more normal kind were observed, and some extension took place. Alkali produced at best only a slight improvement. The cells were here also contracted, they became hyaline and many of them were dissolved. In a m/3 to m/4 Na_2SO_4 solution the character of the cells was similar to that found in a m/2 Na_2SO_4. The contracted granular cells soon became hyaline. Addition of m/100 HCl in the usual proportions did not allow a more marked outgrowth, at least on the first day; the solution was too strong. It is however possible that on the second and third day an improvement would have become apparent as the result of the acid.

Addition of m/200 or m/300 HCl produced a very marked improvement. The outgrowth and character of the cells in this solution resembled that in an acid NaCl solution. The hardening effect of the SO_4 ion was to a great extent neutralized; m/10 to m/30 NaOH also produced in several, but not in all, cases an improvement, the cells became more normal in the alkaline solution. Again alkali favored an early extension and hyalinization and solution of the cells after a relatively short period of preservation.

Effect of a solution of Na citrate. In solutions of 2/3 to m/4 Na citrate no definite outgrowth occurs, but a few round, contracted, granular cells with sharp tongue pseudopodia may be visible around the piece.

Digitized by Google

Addition of m/100 HCl does not cause any improvement. In solutions of 5/12 to 3/12 m Na citrate a few round granular or hyalin contracted cells can be found around the piece whose margin may be partly dissolved.

If 1 part m/2 or 2/3 m Na citrate is added to 9 or 12 parts m/2 NaCl the width of outgrowth, which would occur in the latter solutions, is usually diminished. The cells are very much contracted, round, at first granular, later they become hyalin. The pseudopods are very sharp and often multiple threads. The cells do not readily agglutinate. Added in the same proportions to a m/4 NaCl solution the character of the cells is similar, but in accordance with the lower osmotic pressure of this solution there may be produced some balloon pseudopodia. In combination with m/4 KCl, citrate (added in the same proportions) does not change the character of the cells very much; there occur balloon pseudopods and occasionally even circus movements; neither does citrate distinctly alter the cells in m/2 KCl; perhaps it may increase somewhat the consistency of the protoplasm without noticeably improving the amount of outgrowth. The effect of citrate is thus similar to that of sodium sulphate, but its effectiveness is much greater than that of the sulphate.

In a van't Hoff solution (artificial seawater) no good outgrowth took place. There was usually cytolysis and hyalinization at the margin of the piece. Cells became transformed into radially arranged or arcade like fibers. In three experiments addition of m/100 HCl produced a great improvement; in one of these cases the improvement became definite only on the second day. In three other experiments no improvement was produced.

In sterile seawater (2 experiments) no outgrowth took place; the margin of the piece was dissolved and precipitate formed around it. Addition of m/100 HCl did not call forth any improvement.

In other experiments *Limulus serum was heated to 100°;* the proteid coagulated, and through filtration we obtained a clear fluid. In such filtrate which was free from proteid no or only a slight outgrowth occurred, which was, however, somewhat better than that observed in van't Hoff solution. Addition of acid or alkali in the usual proportions did not lead to a decided improvement.

We see then that a solution whose inorganic composition is similar to that of Limulus serum is much inferior to the latter. This agrees with our previous observations. We found however in experiments carried out in 1920 that occasionally the results obtained in such solu-

Digitized by Google

tions can approach those in Limulus serum. We find here evidently the same variable factor which we noticed in the case of NaCl solution, and again we must leave it undecided, whether this variability depends upon changes in the resistance of the cells or in a variable admixture of substances extracted from the piece.

In a mixture of 2 cc. filtrate of coagulated Limulus serum and 0.2 cc. of m/10 NaCN cell movement took place similar to that observed in the filtrate alone. The cells sent out pseudopods and showed movements of the granuloplasm. These movements are not prevented by cyanid added in the proportions used in this experiments. A similar result was obtained also in other experiments in which NaCN was added to various salt solutions.

The effect of addition of serum to salt solutions. The difference which we found between the action of salt solutions and of Limulus serum suggested the use of a combination of salt solutions and Limulus serum in various proportions, in order to determine how far addition of salt solution to Limulus serum caused a deterioration of Limulus serum and, on the other hand, how far addition of Limulus serum to the salt solution improved the latter. The mixture of serum and salt solution was in each case heated to above 55° for 30 minutes before use.

1. A mixture of a filtrate of Limulus serum heated to 100° and of fresh Limulus serum in the proportion of 3 parts of the filtrate and 1 part of Limulus serum, or of 3 parts fresh Limulus serum and 1 part filtrate acted almost as favorably as fresh undiluted Limulus serum. The second mixture was perhaps not quite so good as the first one, but it approached it very closely.

2. A mixture of 3 parts sterile seawater + 1 part Limulus serum behaved similar to seawater. Addition of serum did not call forth a marked improvement. In one experiment addition of 1 part of seawater to 3 parts of Limulus serum caused a very marked deterioration of the Limulus serum.

3. A mixture of 3 parts of van't Hoff solution + 1 part of Limulus serum was almost as good as pure Limulus serum.

4. In a mixture of 3 or 5 parts of a m/2 NaCl solution + 1 part of serum there was in the majority of cases no marked improvement noticeable over a m/2 NaCl solution; in some cases an improvement was obtained, although the mixture was in no instance as good as Limulus serum. A mixture of 9 parts of a m/2 NaCl solution + 1 part of serum behaved similarly to a m/2 NaCl solution. In a mixture of 5 parts m/4 NaCl with 1 part of serum only a slight increase in the area

Digitized by Google

of outgrowth was noticeable; but the combination of the hypotonic NaCl solution with serum led to the production of balloons and occasional circus movements. This combination had therefore an effect not unlike that of a m/4 KCl solution. An addition of 1 part of a m/2 NaCl solution to 3 parts of serum led to a distinct deterioration of the serum; the result however was usually better than in a m/2 NaCl solution.

5. Through addition of 1 part of a m/2 LiCl solution to 3 parts of Limulus serum a distinct deterioration of the Limulus serum was effected, with exception of one case where the result was as good as in Limulus serum; on the other hand, through addition of 1 part serum to 3 or 5 parts of m/2 LiCl some improvement of the LiCl solution was produced, although the mixture did not become the equal of Limulus serum.

6. Addition of 1 part of a m/2 or m/4 KCl solution to 3 parts of serum produced a marked deterioration of the serum. Typical potassium effects appeared. With m/4 KCl solution in the mixture balloons, circus movements and courts were noted; with a m/2 KCl solution in the mixture circus movements were only occasionally found. The serum caused in a number of cells hyaline extension and it also caused perhaps a slight increase in the outgrowth over that found in KCl solution. It seemed as if addition of serum to a m/2 KCl favored in some cases the appearance of circus movements.

The effect was similar if 1 part of serum was added to 3 or 5 parts of a m/2 or m/4 KCl solution; the typical potassium effects were noticeable. There was an increase in circus movements in m/4 KCl solution, and even in m/2 KCl circus movements were found in some specimens. The effect of serum became noticeable perhaps through a slight increase in outgrowth and hyaline extension of cells which was found in these mixtures.

7. Addition of *1 part of serum to 5 parts of a m/2 or m/4 RbCl or CsCl* called forth an increase in outgrowth, which was very marked in some cases; the effect of RbCl and CsCl was still noticeable in the mixture through the presence of occasional balloons and circus movements.

8. Addition of *1 part of serum to 3 or 5 parts of a m/2 NH_4Cl* solution did not produce a marked change. The cells characteristic of NH_4Cl (multiple drop pseudopodia or flat extended honeycombed cells) could still be found; the outgrowth was slight. The addition of serum may however become apparent in the hyaline spreading out of cells which

Digitized by Google

was found in some cases, and also in the greater number of contracted granular cells. Addition of 1 part of a m/2 NH_4 solution to 3 parts of Limulus serum led to a marked deterioration of the serum. Even here the typical mulberry cells of NH_4Cl were visible.

9. Addition of *1 part serum to 3 or 9 parts of a m/2 or m/3 $CaCl_2$ solution* does not produce a marked change; we still find the marked hyalinization and cytolysis of cells characteristic of $CaCl_2$. Addition of 1 part of $CaCl_2$ to 3 parts of serum usually causes a marked deterioration of the serum and an early cytolysis of the cells.

10. Addition of 1 part of a m/3 $MgCl_2$ solution to 3 parts of serum may give results similar to serum or may produce a deterioration of the serum. Mixtures in which the $MgCl_2$ solution predominated over serum were usually much inferior to serum.

We find then that some improvement may be produced through addition of serum to solutions of single salts, but that usually the result is much inferior to that produced through addition of a trace of acid. If about 25 per cent of salt solution is added to Limulus serum a decided deterioration of the serum usually occurs, but in some cases the result may approach that obtained in pure serum. In mixtures of KCl or NH_4Cl and serum the cellular reactions characteristic of these salt solutions are usually present. In case of certain composite salt solutions, like van't Hoff solution, Limulus serum filtrate which resembles Limulus serum, as far as the inorganic constituents are concerned, addition of 25 per cent of Limulus serum makes the mixtures as good or almost as good as Limulus serum. There exists therefore in Limulus serum a certain surplus of proteids, as far as the protective influence of Limulus serum on cells is concerned.

Effect of hypertonicity of salt solutions on the outgrowth of cells. We have seen that in a 3/4 m NaCl solution the outgrowth is not so good as in a m/2 NaCl solution. This could be directly shown in cases in which the tissues grew well in isotonic solutions of NaCl, while in our recent experiments in which in the majority of experiments the cells proved little resistant to a m/2 NaCl solution, the difference between hypertonic and isotonic solutions became apparent after addition of acid. We found, however, that in the tissues which showed little resistance in 3/4 m and m/2 NaCl solutions, some improvement could be produced through the use of a concentration intermediate between m/2 and 3/4 m. A moderate increase in osmotic pressure seemed to produce an improvement. Solutions intermediate between a 3/4 and 5/8 m solution were usually found best. It is probable that the slightly hyper-

Digitized by Google

tonic solutions retarded the entrance of fluid into the cells and thus exerted a preserving effect. The improvement thus produced was however only moderate and not comparable to that called forth through addition of acid to isotonic NaCl solutions under optimal conditions.

DISCUSSION

Our results indicate that at the isoelectric point amoebocytes are least resistant to injurious influences and have the greatest degree of permeability to the surrounding fluid. We see that acid and to some extent alkali may protect the cells not only in isotonic but in certain cases also in hypotonic and in hypertonic solutions. Under the influence of the acid in optimal concentration the cells, their consistency, the character of pseudopods, the movement of granuloplasm remain healthy. This relation between consistency of protoplasm and character of pseudopods and amoeboid movement is in agreement with our previous conclusions (11).

We may therefore assume that acid prevents the surrounding fluid, salts as well as water, from entering the cells. In hypertonic acid solution an increase in consistency occurs and here we find an increase in the average sharpness of pseudopods. While acid may not prevent the withdrawal of fluid from the cells in hypertonic solutions, it protects to some extent the cells against the entrance and effect of injurious constituents of the surrounding medium. The same protective influence is noticeable to a much greater degree in isotonic and hypotonic solutions. It is very probable that acid acts mainly on the outer layer of the cell; but at present we cannot exclude the possibility that it may in some cases in addition exert a similar effect on inner boundaries between colloid particles and the surrounding liquid. In addition to this protective influence both acid and alkali exert specific effects, acid having an hardening and alkali a softening effect on the cells.

In accordance with this increase in consistency caused by acid pseudopods are on the average sharper in acid than in neutral solutions. The softening effect of alkali leads to a more rapid spreading out and destruction of the cells. The beneficial influence of alkali is therefore usually much more transient than that of acid. The improvement caused by acid or alkali is however limited by the effect of the other substances in the solution. In media which cause such marked alteration of the cells, as we find in solutions of KCl, NH_4Cl and certain other substances, acid and alkali show no or only a very slight beneficial effect.

Digitized by Google

The results we obtained with organic acids indicate that the effects observed in acid solution do not altogether depend on changes in pH concentration, but that the anion may also play a certain part. We find then that in both acid and alkaline solutions the amoebocytes may be active.

Limulus and spider crab blood show according to the measurements of Dr. S. Morgulis a pH of 7.4 to 7.6, a value which does not differ very much from that found in mammalian blood serum.

Within the body and under normal conditions the amoebocytes are therefore suspended in a slightly alkaline medium, and they are, as we have previously seen, on the whole non-motile. It is due to two factors that the cells remain well preserved in this medium: *1*, the action of the proteids of the blood; and *2*, the lack of those stimuli which are active in the changed environment. The normal lining of the body spaces leaves the amoebocytes unchanged; they remain flat, elliptic, non-sticky discs. Some organic constituents of the blood, in all probability the proteids, protect the cells; this follows from the marked difference which is usually found in Limulus serum with its proteids intact and in Limulus serum filtrate obtained from coagulated blood serum or in a van't Hoff solution. The blood proteids protect therefore in all probability the cells for some time against those injurious changes which would otherwise take place at or near the isoelectrical point.

The real pH concentration of the media which surrounded the cells in our experiments remains still to be determined. Substances extracted from the piece of tissue undoubtedly neutralize a part of the acid. So far we have made only a preliminary test with an indicator of the reaction of the fluid which surrounded the piece and which had been made acid through addition of m/100 HCl in the usual proportions. It was still found acid at the end of the experiment. A good outgrowth had taken place in this case.

There may be in addition to the direct effect of acid and alkali an indirect way in which they may perhaps exert a beneficial action. By combining with the proteid material in the fluid protein salts are probably formed which may bind a certain amount of water which would thus be prevented from acting on the blood cells.

It is probable that different kinds of cells and tissues differ in their sensitiveness to acid; it is furthermore certain that a greater concentration of neutral salts counteracts certain injurious effects of acids. Those cells are presumably better able to withstand injurious effects of acid which are better adapted to higher osmotic pressures of the surrounding

Digitized by Google

medium. The earlier investigations of Michaelis and Kramsztyk (12) and the recent studies of Schade, Neukirch and Halpert (13) make it certain that also in mammalians many cells are adapted to a higher hydrogen ion concentration than is normally found in the blood.

In the fluid surrounding the tissues and in the tissues themselves variations occur in the concentration of acid which are caused through the metabolism of the cells. An increase in the acidity of tissues has been held to be the principal cause of edema. From our experiments we may conclude that if a certain degree of hydrogen ion concentration is reached in the fluid directly surrounding the cells the entrance of water into the tissues proper may be prevented. Such an increase in acidity would therefore have rather a preventive than a promoting effect on edema of the tissues in contradistinction to the tissue spaces.

It is probable that what holds good in the case of amoebocytes applies equally in the case of certain other labile cells; in this connection we must however take into consideration differences in the adaptation to osmotic pressure on the part of different kinds of cells; this might modify the effect of acid and alkali on these cells and tissues.

Our experiments very clearly bring out one of the factors which may cause the death of tissue cells. We have previously shown that many mammalian tissue cells have a potential immortality; they die because of unfavorable conditions which develop in their environment.

An analysis of the conditions which lead to the death of cells in the body, and of the factors which tend to preserve the cells, would be of great importance. We know that a low temperature which diminishes metabolism prolongs the life of cells and organisms. In the case of tissues this preserving influence has been used in many instances; but it has also been shown by Jacques Loeb and Northrop (14) and Raymond Pearl (15) to hold good in the case of whole organisms (Drosophila). Here the duration of life is, as these authors have shown, a function of the intensity of the metabolism and therefore of the temperature. In the eggs of Arbacia Jacques Loeb could increase the resistance of the cells to injurious influences through withdrawal of oxygen (16).

In the case of the blood cells of Limulus we recognized another factor as injurious and causing processes of dissolution, namely, the spreading out of the cells in response to stereotropic stimuli (10). A cell which in the contracted state might be preserved even in a not fully adequate medium, becomes dissolved as soon as it begins to spread out, or even with increased intensity, after it has become fully extended. If we pour

Digitized by Google

very gently on amoebocytes, which are firmly or loosely fixed on the surface of a slide, a fluid which has a moderately injurious influence on the cells, we notice that it is always the extended cells which are dissolved first, while the contracted cells resist much longer the injurious effect of the solution. We may then conclude that in all probability the process of spreading out leads to an increase in the permeability of the cells which now become more readily accessible to the injurious effect of water and other constituents of the surrounding medium which tend to enter the cells. By delaying the spreading out of the cells through various means, for instance, through placing the tissue on a base of vaseline, or paraffin, or through increasing the consistency of the cells through a combination of salts with acid or alkali, or perhaps through a slight increase in the osmotic pressure of the surrounding medium, we may be able to delay the spreading out of the cells and thus in favorable cases to prolong the life of the cells over a considerable period of time.

In this connection we wish to point out that the stereotropic spreading out of cells is a phenomenon of considerable importance in tissue formation in general, and we must consider the possibility that an increase in the permeability, which we found associated with the process of spreading out in the case of amoebocytes, may under similar conditions also occur in other cells and perhaps play a part in tissue differentiation.

SUMMARY AND CONCLUSIONS

1. The effect of various salt solutions on the cells emigrating from cultures of experimental amoebocyte tissue accords with the effect of these solutions, formerly described by us, on blood cells suspended in a drop of blood which has been received directly in these solutions. We found a decrease in consistency of the cells under the influence of potassium salts, a production of pseudopods followed by hyalinization and cytolysis in sodium chloride, an intermediate condition in RbCl and CsCl, a preservation of granules and the formation of mulberry cells in NH_4Cl, a marked hyalinization and cytolysis in $CaCl_2$, a contraction and increase in consistency of cells in Na_2SO_4 and citrate solutions. In accordance with our former investigations we found that all those solutions which tend to decrease the consistency of the cells cause a broadening and rounding off of pseudopods (hypotonic solutions, salts of K, Rb, Cs). While an increase in consistency causes the pseudopods to be finer and sharper (hypertonic solutions, sulphates, citrate); in NH_4Cl multiple rudimentary droplet pseudopodia may appear.

Digitized by Google

Through removal of the proteids from the blood serum a deterioration is caused in the Limulus serum; addition of blood serum to the filtrate of coagulated Limulus serum in proportions of 1 part of serum to 3 parts of filtrate or van't Hoff solution restores the efficiency of the latter as a medium for the blood cells. Addition of blood serum to solution of single salts, which are in themselves injurious for the blood cells, may produce an improvement, without however producing an effect equal to that seen in pure blood serum. Addition of as little as 10 per cent serum to the salt solution is without marked effect.

We found a considerable variability in the effect of solution of NaCl on cells emigrating from amoebocyte tissue; while in some cases the outgrowth was as good as in Limulus serum, in others a rapid hyalinization and cytolysis occurred. This effect of NaCl solutions on the amoebocyte tissue would be in accordance with the direct effect of NaCl on isolated amoebocytes. It remains to be determined whether this variability in the effect of NaCl solution on cells emigrating from amoebocyte tissue is due to variations in the resistance of the amoebocytes in different animals, or, to an unequal admixture of substances extracted from the piece of tissue.

2. Addition of acid and alkali in certain proportions causes not only a marked improvement in the outgrowth of the cells, provided the injury caused by the salt solutions to which acid or alkali are added has not exceeded a certain limit, but the outgrowth in acid solutions may definitely surpass that in Limulus serum, which otherwise represents the most favorable medium for the amoebocytes of Limulus. Near the isoelectric point solutions seem to be most injurious as far as the preservation, as well as the activity, of the amoebocytes is concerned. The beneficial effect of acid and alkali may be noticeable even in hyper- and hypotonic solutions. Acid and alkali probably decrease the permeability of the cells, or of certain constituents of the cells, and thus save the cells from the injurious effect of water and substances dissolved in the water, which otherwise would change the colloids of the cells. Especially acid increases the contraction and consistency of the cells and accordingly also the sharpness of the pseudopods.

3. Both organic and inorganic acids are effective in concentrations which do not differ very much; it is therefore probable that the effect of the acid is not altogether due to changes in hydrogen ion concentrations which it produces.

4. In addition to the preserving action common to acid and alkali, both exert specific effects on the amoebocytes, in alkali the extension

Digitized by Google

and subsequent solution of cells taking place much more rapidly than in acid; the former have a softening, the latter a hardening effect upon the cells and their membranes.

5. Certain organic constituents of the Limulus blood serum, probably its proteids, exert a protective influence upon the amoebocytes; this constituent, however, is not so effective as acid in optimal concentration.

6. The stereotropic extension of the amoebocytes leads to degenerative changes in these cells, probably as the result of an increase in the permeability caused by the process of spreading out. Conditions which counteract this spreading out of the cells tend therefore to prolong their life for a considerable period of time, provided these conditions are not otherwise injurious. The spreading out of amoebocytes, which we observe in vitro, is analogous to the extension of somatic cells of higher organisms during embryonic development. In both cases the extension leads to the production of tissue-like structures.

We wish to express our thanks to Miss E. M. Paxson for her assistance in these experiments.

BIBLIOGRAPHY

(1) Leo Loeb: Univ. Penna. Med. Bull., May, 1905; Folia Haematol., 1907, iv, 313; Pflüger's Arch., 1910, cxxxi, 465.
(2) Michaelis and Takahashi: Biochem. Zeitschr., 1911, xxix, 439.
(3) Jodlbauer und Haffner: Pflüger's Arch., 1920, clxxix, 121; Pflüger's Arch., 1920, clxxix, 140, 144.
(4) Hoeber und Spaeth: Pflüger's Arch., 1914, clix, 433.
(5) Jacques Loeb: Pflüger's Arch., 1899, lxxv, 308.
(6) Lillie: This Journal, 1906, xvii, 89.
(7) Jacques Loeb and Wasteneys: Biochem. Zeitschr., 1911, xxxiii, 489; 1912, xxxix, 167.
Jacques Loeb: Biochem. Zeitschr., 1912, xlvii, 127; Journ. Biol. Chem., 1915, xxiii, 139.
Jacques Loeb and Cattell: Journ. Biol. Chem., 1915, xxiii, 41.
(8) Osterhout: Journ. Biol. Chem., 1914, xix, 335, 493, 517.
(9) Collett: Journ. Exper. Zoöl., 1921, xxxiv, 75.
(10) Leo Loeb: Washington Univ. Studies, 1920, viii, Scientific Series, 3.
(11) Leo Loeb: This Journal, 1921, lvi, 141.
(12) Michaelis and Kramsztyk: Biochem. Zeitschr., 1914, lxii, 180.
(13) Schade, Neukirch und Halpert: Zeitschr. f. d. gesammt. Exper. Med., 1921, xxiv, 11.
(14) Jacques Loeb and Northrop: Journ. Biol. Chem., 1917, xxxii, 103.
(15) Pearl: Sci. Monthly, August, 1921, 143.
(16) Jacques Loeb: Biochem. Zeitschr., 1906, ii, 81.

Digitized by Google

STUDIES ON THE PHYSIOLOGY OF GASTRO-ENTEROSTOMY

I. The Regurgitation of Intestinal Contents in Normal Dogs and Dogs with Posterior Gastro-enterostomy

G. E. BURGET and M. E. STEINBERG

From the Department of Physiology, University of Oregon Medical School

Received for publication December 21, 1921

Different investigators have in the past few years reported finding duodenal juices in the normal stomach, especially when the acidity there was of fair degree. Boldyreff (1) studied the circumstances by which duodenal contents might be expected to be found in the stomach. He found that this reflux took place when the acidity of the stomach was higher than the duodenal mucosa could tolerate, a mechanism which tended to keep the acidity of the stomach at a low level. He called this mechanism the self-regulation of the acidity of the stomach contents. His work was done on dogs with gastric fistula. Morse (2) found that regurgitation often occurred in anesthetized pithed dogs when the acidity reached 0.2 per cent and nearly always with acid of higher concentration. He also found that the higher the concentration (0.1 to 0.5 per cent) of acid the greater the regurgitation. The rate of emptying of the stomach decreased with the increase of acidity. Hicks and Visher (3) introduced by means of a tube 150 cc. of 0.5 per cent HCl into the stomach of dogs and noted a regurgitation in fifteen minutes in 30 per cent of the tests and in thirty minutes in 45 per cent.

They state that the introduction of 0.4 per cent HCl in a human subject showed no regurgitation at the end of twenty minutes. A psychic secretion averaging 32.6 cc. (acidity 0.411 per cent) obtained in an individual with a gastric fistula caused a reflux in 40 per cent of ten trials. Rehfuss and Hawk (4) assert that the introduction of 0.5 per cent HCl in a human subject caused regurgitation of the alkaline intestinal secretion. Moppert (5) reported spontaneous reflux of bile in 72 per cent of cases examined using the Einhorn thread. Jarno (6) found regurgitation in the human stomach after introduction of relatively weak acid solution, 0.08 per cent.

Digitized by Google

We thought it worth while to repeat Boldyreff's experiments on normal dogs, using a stomach tube for introducing the acid and aspirating samples and then to make a similar study of these animals after gastro-enterostomy to learn the effect on the normal mechanism of gastro-enterostomy. We had no difficulty whatsoever in using the stomach tube. After the first few times the tube was introduced the dogs showed no signs of discomfort and did not struggle. We were surprised to see the ease with which the test could be carried out.

Dogs of average size and in good condition were chosen. They were kept in the laboratory during the period of normal test but were given exercise outside every other day. They received no food for twenty-four hours previous to the test but were allowed water at all times. One hundred to 150 cc. of 0.5 per cent HCl were given with a stomach tube, the dog was then allowed to get on his feet and walk about for a minute or two, giving opportunity for the acid introduced to mix with the stomach contents. A sample was then obtained by aspiration and titrated. The presence of water, if any was in the stomach, was shown by this titration. A second sample was taken fifteen minutes after introduction of the acid, another in thirty minutes, etc., until the acidity was reduced to 0.1 to 0.2 per cent, or the stomach empty. The bile coloration was used as an indication of a reflux from the duodenum.

Our results on the normal dogs confirm the findings of Boldyreff. The average length of time for reduction of the acidity to 0.1 to 0.2 per cent was in our experiments seventy-five minutes to ninety minutes. The first appearance of bile varied considerably with different dogs and different experiments. It was sometimes present in fifteen minutes, often in thirty minutes and a few times was delayed for an hour.

Paterson (7) demonstrated the presence of bile in the gastric contents by Gmelin's reaction in 73 per cent of his patients after gastro-jejunostomy. He also stated that the total acidity was reduced 30 per cent, partly due to the neutralization of the acidity by the bile and pancreatic secretion and partly to earlier stimulation of the pancreas bringing about an earlier diminution of the gastric secretion (8). Lemon (9) found a reduction of the acidity after gastro-jejunostomy of 39 per cent total and 46 per cent free. A study of two hundred cases was made and the reduction ascribed to flowing into the stomach of the alkaline secretion of the duodenum.

The gastro-enterostomy opening in our dogs was made 2½ to 3 cm. long on the posterior wall of the antrum of the stomach near the pylorus. The opening in the duodenum was made at the tail of the pancreas.

Digitized by Google

Care was exercised in feeding for a few weeks to permit complete healing about the stoma. In no case were there untoward symptoms following the operation. All the animals made a speedy recovery and soon regained any loss of weight. Allowing two or three weeks for recovery the tests were again made as before the operation. Bile was often present in the first sample, the one taken almost at once after the acid was given. It was regularly present after fifteen minutes and the process of partial neutralization was carried on with corresponding rapidity. After thirty to forty-five minutes the acid was reduced to 0.1 to 0.15 per cent and the stomach nearly empty.

Whether or not after gastro-enterostomy the acid brings about an earlier stimulation of the pancreatic secretory mechanism or the mechanism for regurgitation is rendered more efficient, that is, the stoma permits a freer flow of intestinal juices into the stomach, we are unable to say. Cannon (10) has shown that the stoma may act as a valve, letting contents pass into the stomach but closed in the other direction, especially when the stomach is distended. Since there was a shorter emptying period in our experiments after gastro-enterostomy it must be accounted for in one of two ways. Either the earlier neutralization allowed passage sooner through the pylorus or a part of the contents passed through the gastro-enterostomy opening. These questions are being investigated.

The manner in which the duodenum forces back its contents as reported by Boldyreff (1) is by antiperistalsis. Hicks and Visher (3), however, claim not to have seen any antiperstaltic movements in their experiments but rather constriction bands. This point will be reported on with further studies in this field.

SUMMARY

1. The alkaline juices of the duodenum are regularly regurgitated into the stomach of the dog within thirty to forty-five minutes after the introduction of 100 to 150 cc. of 0.5 per cent HCl.

2. After introduction of 100 to 150 cc. of 0.5 per cent HCl, the acidity of the stomach contents is reduced to 0.1 to 0.15 per cent in seventy-five to ninety minutes and the stomach empties at a rate in proportion to the rate of regurgitation of duodenal juice.

3. In dogs with posterior gastro-enterostomy duodenal regurgitation takes place within fifteen minutes after introduction of 100 to 150 cc. of 0.5 per cent HCl and the acidity of the stomach contents is reduced to 0.1 to 0.15 per cent in thirty to forty-five minutes.

Digitized by Google

Experiments on a number of animals gave concordant results. Typical experiments on two animals before and after posterior gastro-enterostomy are shown in the table.

TABLE 1

150 cc. of 0.5 per cent HCl introduced

TIME INTERVALS	NORMAL		AFTER GASTRO-ENTEROSTOMY	
	Total acidity per cent	Character of specimen	Total acidity per cent	Character of specimen
Dog 7				
minutes				
2	0.4466	Clear	0.4922	Turbid
15	0.4102	Clear	0.2918	Bile ++
15	0.3646	Bile +	0.2553	Bile ++
15	0.3554	Bile +	0.1824	Bile +++
15	0.3007	Bile ++	0.1185	Bile +++
15	0.1824	Bile +++		
Dog 4				
2	0.4375	Clear	0.4740	Bile, trace
15	0.3646	Bile, trace	0.3098	Bile ++
15	0.3281	Bile, trace	0.1454	Bile +++
15	0.2644	Bile ++	0.06	Bile ++++
15	0.2553	Bile ++		
15	0.1915	Bile ++		

BIBLIOGRAPHY

(1) Boldyreff: Ergebn. d. Physiol., 1911, xi, 119.
(2) Morse: This Journal, 1916, xli, 439.
(3) Hicks and Visher: This Journal, 1915, xxxix, 1.
(4) Rehfuss and Hawk: Amer. Journ. Med. Sci., 1920, clx, 428.
(5) Moppert: Presse Med., Paris, 1921, xxix, 415.
(6) Jarno und Vandorfy: Deutsch. Med. Wochenschr., 1921, xlvii, 381.
(7) Paterson: Trans. xvii, Internat. Cong. Med., Surg. Sect., London, 1914.
(8) Paterson: Journ. Amer. Med. Assoc., 1907, xlix, 657.
(9) Lemon: Northwest Med., 1919, xviii, 179.
(10) Cannon and Blake: Ann. Surg., 1905, xli, 686.

Digitized by Google

THE PULSE IN THE PORTAL VEIN[1]

H. FEIL AND D. D. FORWARD

From the Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio

Received for publication December 24, 1921

The portal vein occupies a unique position in the anatomy of mammals, lying, as it does, between two great sets of capillaries, those of the splanchnic region on the one hand and those of the liver on the other. Therefore the inflow of blood into the portal system must be controlled to a large extent by the caliber of the splanchnic vessels, while the outflow must likewise be controlled by the caliber of the liver vessels. Many conditions might disturb the equilibrium of this double-ended regulation as, for instance, variable vasomotor activity, changes in heart rate and blood pressure, or even changes in vena cava pressure. Since resulting variations in blood flow must react on certain activities of the liver cells, the importance of the portal circulation becomes at once apparent.

This importance combined with the fascination of the unusual regulatory mechanism of the portal blood flow, especially in its relation to liver volume, has attracted many investigators, but no one appears to have inquired into the nature or importance of the pulse variations taking place during each cardiac cycle. Indeed it might well be suspected that such a pulsation does not exist, inasmuch as the pulse wave tends to be abolished completely in its passage through the splanchnic vessels. Such, however, is not the case, for we found in our experiments on dogs a cardiac portal pulse of definite contour.

By recording this cardiac pulse graphically and by studying the changes in its contour under various conditions, a considerable knowledge was obtained of the portal mechanism—how, at times, the outflow of blood exceeded the inflow until equilibrium became established while, at other times, just the opposite occurred and inflow exceeded outflow.

Anatomy of the portal vein. A diagram of the portal system of the dog may be found in an article by Burton-Opitz (1). The main trunk is

[1] This investigation was aided by a grant from the American Association for the Advancement of Science.

Digitized by Google

formed by the union of the superior and inferior mesenteric veins. About 2 cm. cephalad from this juncture, the vein is joined by the gastro-splenic branch. The portal vein in the dog is 4 to 6 cm. long and 6 to 8 mm. in diameter when distended with blood.

Method. Dogs of medium weight were carefully anesthetized with morphine and chloretone, and throughout the experiments were permitted to breathe normally. Through the gastro-splenic branch a small curved cannula 27 mm. long and 2 mm. in diameter was introduced into the portal vein, the open end of this cannula being turned toward the splanchnic capillaries. The distance from the mouth of the cannula to the capillaries forming the inferior mesenteric vein (capillaries of the large intestine) averaged 8 cm. while the distance from the cannula to the capillaries forming the superior mesenteric vein (capillaries of the small intestine) was about 20 cm. On the insertion of the cannula the gastro-splenic vein had to be tied, thereby causing congestion of the spleen and a small portion of the stomach. Although within the lumen of the portal vein, the cannula, because of its small diameter, interfered but little with the passage of portal blood.

The cannula was rigidly connected to a Wiggers' optical manometer (2) covered by light rubber dam, in order to make it sensitive. The cannula of a similar manometer, equipped with a heavier rubber membrane was inserted into the abdominal aorta at the forking of the iliacs. Heart sounds were recorded simultaneously. A tambour, held to the shaved chest of the animal by an elastic belt was connected by rubber tubing to a Wiggers' heart sound segment capsule (3). A tuning fork vibrating fifty times a second gave the time measure.

Thus accurate and simultaneous records of the heart sounds, abdominal aortic pulse, and portal pulse were taken.

The contour of the portal pulse. The pressure variations in the portal vein really represent changes in the volume of blood contained in that vein with certain modifications introduced by extra-portal mechanical causes. Figure 1 shows a typical record of the portal pulse taken simultaneously with records of heart sounds and abdominal aortic pulsations. The heart rate in this record was 182 per minute, a rate not unusually rapid in the dog, so we may consider this record as showing the normal portal pulse of the dog.

In general, the curve falls during systole and rises to a rounded summit during diastole. The systolic fall, however, is interrupted by a sharp peak which occurs almost simultaneously with the peak of the abdominal aortic pulsation and this is followed by a sharp decline.

Digitized by Google

With greater detail, we may describe the curve as follows: At the exact instant that systole begins (as indicated by the large vibration of the first heart sound) the curve has already begun to decline gradually. This gradual decline, however, is interrupted by a sharp peak, *a-b*, after which it falls abruptly to a trough, *c*. Then the upstroke of the main wave, *d*, commences. The beginning of this rise precedes slightly the early vibrations of the second heart sound; in other words, it begins in late systole and is continued well into diastole so that the crest of the

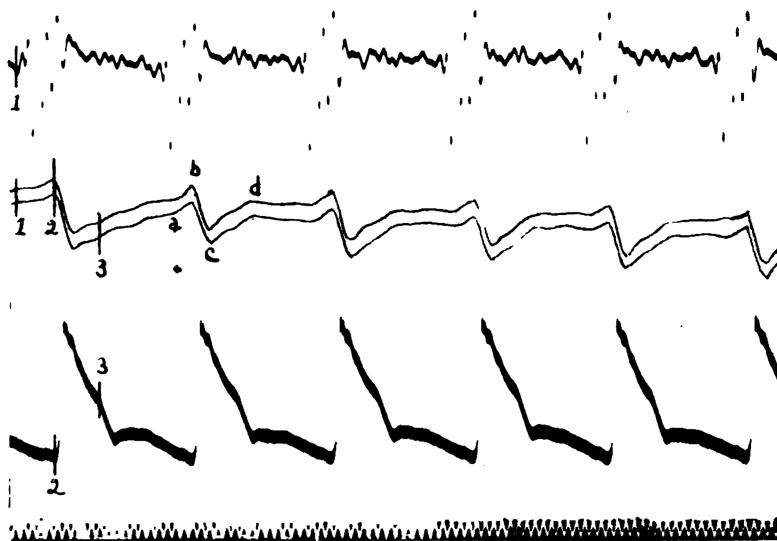


Fig. 1. Simultaneous records of apex sounds, portal pulse, and abdominal aortic pulse. Time relations: *1*, beginning of systole of heart; *2*, time that ejection wave of arterial pulse appears in abdominal aorta; *3*, end of systole waves *a*, *b*, *c* and *d* referred to in text.

curve is approximately mid-diastolic. From the crest, the curve declines slowly until the next systole occurs and the cycle is repeated. It should be pointed out that neither the amplitude nor the duration of the systolic peak, *a-b*, was always as pronounced as in this illustration. In some animals the rise *a-b* was barely indicated, but in all cases the sharp depression, *b-c*, was clearly shown. In some of our records no respiratory variations of pressure curves were found; in others, however, cardiac pulsations were superimposed on more gradual variations synchronous with respiration. These changes caused by respiration

Digitized by Google

consist in a considerable fall in the level of the entire curve at inspiration and a relatively steep rise during expiration. The contour of the wave seen in the cardiac portal pulse remains approximately constant during this respiratory fall and rise in general pressure. In normal curves a level stretch follows the fall in pressure.

It is difficult to determine the exact reason for these respiratory variations. Their abruptness and close relation to phases of inspiration and expiration suggests a mechanical cause, possibly a movement of the abdominal viscera due to the descent of the diaphragm rather than a direct effect of varying intrathoracic pressures on the filling and emptying of the portal system.

The effect of slowed heart-rate. The human heart is only about one-half as rapid as that of the dog. To more nearly approach the actual condition in man, therefore, it became necessary to study the portal pulse when the dog's heart rate was slowed sufficiently to lie within human range. To attain this end, the right vagus was sectioned and the peripheral end stimulated by a mild faradic current.

With such stimulation the general level of the portal curve falls rather abruptly (fig. 2). If the diastolic pause is greatly prolonged, the curve drops to its minimum level in one beat. If the diastolic periods are not lengthened so much, this fall in portal pressure takes place over several beats. With maintenance of vagal stimulation, the curve continues to be inscribed at its lowest level. This change of general pressure level due to the slowed heart indicates that the minute volume entering the portal system is reduced more than the efflux out of the liver. With removal of vagus stimulation the curve rises to its normal level in steplike fashion during several beats.

On casual examination, the contour of the cardiac portal pulse appears to change from normal when the heart is slowed (fig. 3). The most obvious difference is that the summit of the *d* wave is followed by a more prolonged diastolic fall not present in rapid rates normal to our dogs. The entire wave consequently appears of larger amplitude, its summit being either rounded or flat. Time relations show, however, that the summit *d* occurs approximately at the same time after the end of systole. The fact that it is no longer in mid-diastole is entirely due to the greater prolongation of diastole which gives opportunity for a subsequent diastolic fall to express itself more clearly.

Effect of intravenous saline infusion. Inasmuch as the arterial blood pressure was somewhat reduced due to unavoidable manipulation of the intestine, it was questionable whether our so-called normal curves

Digitized by Google

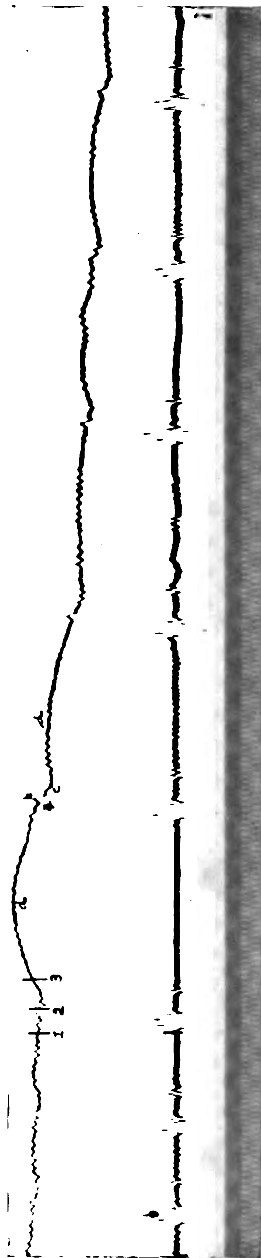


Fig. 2. Record of portal pressure and heart sounds showing cardiac slowing causes reduction of general level of portal pressure and an increasing pressure of the *d*-wave. (Very much reduced in reproduction.)

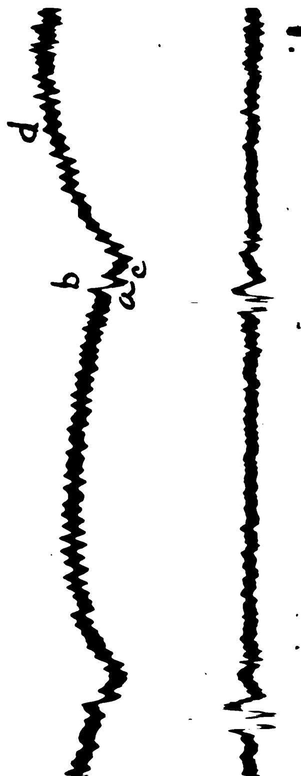


Fig. 3. Portal pressure waves during vagal slowing (less reduction).

Digitized by Google

really represented the true normal after all. We therefore determined to maintain an effective systolic discharge of the heart by means of saline infusion in an effort to ward off the changes of circulation inaugurated by the pre-shock state. Normal salt solution was therefore introduced slowly into the right jugular vein in quantities varying from 600 to 1000 cc. per dog. As the general level of the portal pressure rises during each infusion, the details of the cardiac pulsations become more distinct. As shown in figure 4, the fall during systole is more abrupt, broken as usual by an unchanged peak, *b*. The diastolic ascent is steeper and the summit, *d*, higher. As compared with the normal rather flat curve, the wave under infusion appears distinctly rounded. From the details so far analyzed, we may estimate approximately the contour of the portal pulse in unoperated animals with slow and rapid hearts. In man, we believe it will closely resemble the contour of a dog's portal pulse under saline infusion and slowed heart rate.

Interpretation. In endeavoring to interpret the cardiac variations in portal pressure, it is important to bear in mind that the portal pressure at any moment is determined by the balance between the inflow of blood through the splanchnic capillaries and the outflow through the liver capillaries. Whenever the portal pressure is on the increase, inflow exceeds outflow and vice versa whenever the portal pressure declines, outflow exceeds inflow.

With every systolic increase of pressure in the tributaries of the mesenteric arteries a larger pulse volume is sent through their capillaries into the portal vein. Owing to the relatively great capillary resistance the entrance of this volume pulse into the portal system is somewhat delayed. This accounts, we believe, for the elevation of pressure late in systole and its continued elevation in early diastole (*c-d*, fig. 4). Occasionally, as in figure 1, a stable equalized condition obtains for a short while, accounting for the flattened summit of *d*. Then follows a state where outflow exceeds inflow, due, no doubt, to the fact that the portal vein receives a smaller quantity of blood as the arterial pressure falls during diastole. Consequently, the portal pressure then falls definitely during the remaining portion of diastole. If the heart is rapid, diastole is short and the next systole supervenes before this fall has had time to express itself. Consequently, as in figure 1, the pressure continues to rise almost throughout diastole and falls during systole. Were these the only factors determining portal pressure variations during systole and diastole, we should expect the curve to show an unbroken fall during early systole. Actually we find a small peak, *a-b*, followed by a sharp

Digitized by Google

drop, *b-c*, at the beginning of systole. As shown in actual records, the elevation *a-b* occurs 0.04 to 0.06 of a second after the first sound, while the depression, *b-c*, begins slightly before or with the rise of pressure in the abdominal aorta at its bifurcation. In the interpretation of these sharp variations, *a*, *b* and *c*, it is difficult to conceive of any sudden change in outflow or inflow sufficiently rapid to cause this acute change in pressure. It might be suggested that there is blocking of the portal outflow at the juncture of the portal and hepatic tributaries by higher pressure in the hepatic artery. If this were the case, however, we should expect an increase of pressure throughout systole and not a sharp decrease following the sudden rise. Furthermore the rise, *a-b*, apparently occurs

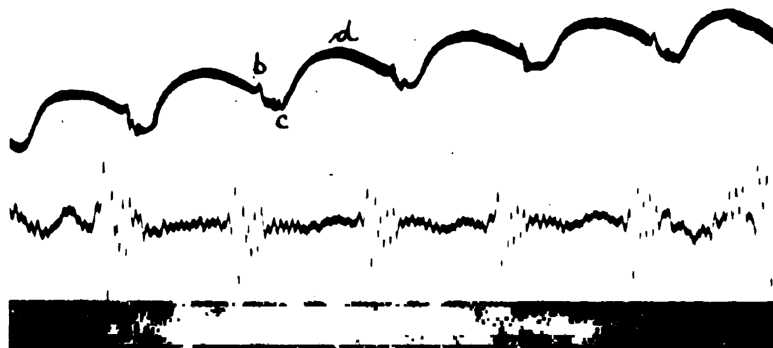


Fig. 4. Portal pressure curves (rapid heart) and heart sounds during moderate saline infusion, showing augmentation of the main wave, *d*.

before the pulse in the abdominal arteries, and precisely when such an effect may be looked for, viz., at point *b* the portal pressure drops, i.e., changes in a direction the reverse of that anticipated. It is also conceivable that the peak and subsequent fall is similar to the arterial impact recorded in jugular tracings, or that it is caused by the transmitted pulsation of the heart through the diaphragm. After mature consideration of all these and other possibilities in relation to the time of their occurrence, we believe that the rise and fall of the *a-b-c* wave may be most satisfactorily explained as due to an impact and traction effect of the following kind: Since the rise *a-b* often occurs before the ejection phase has started, and as it varies in amplitude in different animals, we interpret this as a mechanical impact given by the change in cardiac

Digitized by Google

position transmitted through the diaphragm and liver to the portal system. Since the sharp decrease *b-c* occurs practically simultaneously with the aortic pulsation, it appears that abdominal arterial pulsations in some way exert a slight traction effect on the portal system, and produce this sharp decrease in pressure. The close proximity of many large arteries to the portal vein makes such an explanation probable.

TABLE 1

B-C INTERVAL IN SECONDS		HEART RATE PER MINUTE		PRESSURE CHANGE
Normal	Peripheral vagus stimulation	Normal	Peripheral vagus stimulation	
0.06	0.06	192	96	–
0.06	0.07	182	59	–
0.02	0.02	240	83	–
0.025	0.025	240	150	–
0.02	0.03	207	115	–
0.052	0.054	208	54	–
0.07	0.07	142	66	–
0.052	0.058	186	72	–
0.06	0.07	186	53	–
0.056	0.58	192	102	–
0.034	0.038	144	87	–
Normal	Central vagus stimulation	Normal	Central vagus stimulation	
0.04	0.06	186	180	–
0.02	0.036	222	222	–
0.06	0.09	142	124	–
0.05	0.062	186	182	–
Normal	Epinephrin injection	Normal	Epinephrin injection	
0.052	0.07	139	68	–
0.058	0.078	146	153	
0.034	0.04	186	168	–
Normal	Amyl nitrite inhalation	Normal	Amyl nitrite inhalation	
0.054	0.04	139	150	+
0.04	0.02	184	196	+
Normal	After saline infusion	Normal	After saline infusion	
0.052	0.056	208	216	
0.058	0.058	158	130	

If the peak, *b*, represents the beginning of an extraneous effect due to an arterial pulsation and the rise at *c* indicates the arrival of the systolic volume pulse through the splanchnic capillaries, then the time required for the volume pulse to travel through the mesenteric arteries and their

Digitized by Google

capillaries can be calculated by this interval. Such calculations of this *b–c* interval have shown that point *c*—the beginning of the rise of the diastolic wave—is from 0.02 to 0.07 of a second after *b* depending undoubtedly on the size of the animal and on circulatory conditions in the splanchnic vessels. It may be seen, however, from the tabulated results that this interval is rarely less than 0.04 of a second and, furthermore, that it is not affected by changes in systemic blood pressure such as are produced by cardiac slowing, nor was it materially affected by such volumes of saline as were infused during our experiment.

Evidence favoring our interpretation of curves. A substantiation of our interpretation of the portal pulse waves is found in experiments in which the resistance of the splanchnic capillaries was experimentally altered. If the splanchnic arterioles are contracted in any way, we should expect a greater delay in the transfer of a pulse volume into the portal veins, i.e., the *b-c* interval should lengthen. Furthermore, portal inflow being

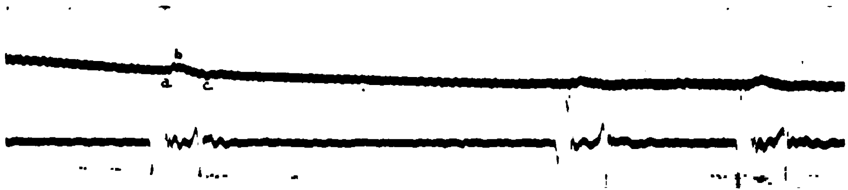


Fig. 5. Portal pulse curve and heart sounds during action of epinephrin. Note practical abolition of all portal pulse waves except small variations, *a-b; b-c* interval increased, *d*-wave entirely absent.

diminished and outflow continuing, we should expect the entire *d* wave to be of smaller amplitude. If, on the other hand, the splanchnic arterioles are dilated, the reverse effects viz., a shortening of the *b–c* interval and an increased amplitude of the d-wave, would be expected.

An increased splanchnic resistance was brought about by central vagus stimulation. With no change in heart rate, the general level of the portal pressure curve declined, thus demonstrating the reduced minute volume of blood reaching the portal vein. Similar but more intense vasoconstriction was also produced by experiments in which epinephrin was injected. Again there was a prompt and profound fall in the general portal pressure. Under moderate vasoconstriction thus produced, the wave *d* became much smaller while during intense epinephrin constriction, it disappeared entirely (fig. 5). As the epinephrin effect wore off this wave very gradually reappeared. While the chief wave, *d* in the portal pulse disappeared under profound epinephrin

Digitized by Google

effect, the wave *a-b* caused by the mechanical impact of the heart persisted, unaltered in contour. With these variations in contours, the anticipated effects on the duration of the *b-c* interval were found. As shown in the table, both after reflex vasoconstriction induced by central vagus stimulation and after splanchnic constriction from epinephrin, the *b-c* interval is materially lengthened. Contrary effects were observed in experiments in which the splanchnic vessels were dilated by inhalation of amyl nitrite. The general pressure curve then increased. The *d* wave increased in amplitude and, as shown in the table, the *b-c* interval was definitely shortened.

SUMMARY

1. A portal pulse consisting of two waves is produced in the portal vein during each cardiac cycle.

2. The first wave, *b*, we interpret as due to extraneous impact and traction, the second or real wave, *d*, we believe due to the changing balance which occurs between inflow and outflow of the portal system, at different times of the cardiac cycle.

3. Our interpretation of the d-wave is as follows: Following each systolic ejection a certain pulse volume reaches the splanchnic vessels. Owing to the splanchnic resistance there is a delay of 0.02 to 0.07 of a second (average 0.04 of a second) in the transference of this increased volume to the portal vein. Consequently, the portal pressure begins to rise later than the aortic pulse by this interval. This transfer of blood does not cease with the end of systole but continues into diastole, consequently, portal pressure reaches its maximum relatively early in diastole. At this stage, the splanchnic inflow exactly equals hepatic outflow and wave *d* has reached its summit. Thereafter outflow exceeds inflow and portal pressure declines.

4. The amplitude of the chief or *d* wave is determined by *1*, the length of diastole; *2*, by the pulse volume entering the portal vein. The latter is determined by *a*, the systolic discharge of the left ventricle; and *b*, the degree of splanchnic constriction or dilation.

5. Evidence is submitted that the rise of the *b* wave is probably due to a transmitted cardiac impact while the sharp drop is attributed to an arterial traction.

6. According to this interpretation of the *b* and *c* waves, the *b-c* interval represents the time required for the systolic pulse volume to be transferred from the splanchnic tributaries to the portal vein. This interval is unaffected by changes in heart rate or moderate saline infu-

Digitized by Google

sion but is decreased whenever the splanchnic vessels are constricted, and increased whenever they are dilated.

The direction and guidance of Prof. Carl J. Wiggers in this investigation is gratefully acknowledged.

BIBLIOGRAPHY

(1) Burton-Opitz: Pflüger's Arch., 1910, cxxxv, 206.
(2) Wiggers: Circulation in health and disease, Philadelphia, 1915, 51.
(3) Wiggers: Amer. Journ. Med. Sci., 1917, cliii, 666.

Digitized by Google

BLOOD PRESSURE RESPONSES TO HYPERSYSTOLIC COMPRESSION OF TISSUES

K. A. MARTIN AND H. L. WHITE

From the Physiological Laboratory of Washington University, St. Louis

Received for publication December 27, 1921

The present work was begun as a study of the effect of smoking on blood pressure. This involved an attempt to follow qualitatively blood pressure variations continuously over considerable periods and to record these graphically with special apparatus. It was during the course of these observations that several phenomena presented themselves, an investigation of which forms the basis of the work presented here.

Apparatus and principle. We may first describe the apparatus and methods employed in the original problem, i.e., the effect of smoking on blood pressure. Airtight aluminum jackets covering the hand and about two-thirds of the forearm were cast from plaster molds. To make the jackets airtight they were impregnated with paraffin and removable rubber gaskets were laid along the approximating flanged edges of the two halves, which were held tightly together by bolts. A rubber cuff fastened to the proximal end of the jacket made a tight junction between jacket and upper forearm. Air pressure was applied in the jackets and piston recorders were connected with them through an Erlanger sphygmoscope to record the oscillations of the pressure.

The principle that a rise in blood pressure manifests itself by an increase in the amplitude of pulsations transmitted through a cuff set in the neighborhood of systolic pressure and by a decrease in the amplitude of pulsations transmitted through a cuff set in the neighborhood of diastolic pressure, the reverse occurring in connection with a fall of blood pressure (1), (2), was employed. This method has been in use in this laboratory for the past five years; work on its quantitative standardization is now in progress. As used here it reveals only qualitative changes, i.e., whether the blood pressure rises or falls, but does not give quantitative results. Jackets were used instead of cuffs with the

Digitized by Google

idea that they would be much less painful when the compression was to be maintained for some time, and this supposition was verified by experience. Only a slight local discomfort was experienced during a compression lasting 40 to 60 minutes. The term "hypersystolic jacket" will be used in reference to the jacket by which the compression above systolic blood pressure was applied; "hypodiastolic jacket" for the one by which compression slightly below diastolic was applied.

Method. The method of procedure in the first set of experiments was to take the subject's blood pressure by the auscultatory and graphic methods, the Erlanger instrument being used. The pressure in one jacket was then set about 10 mm. Hg. above systolic and that in the other at about or a little below diastolic. After a few minutes the subject began to smoke a cigar. Mild cigars were used and were smoked in a leisurely manner. The smoking was usually continued until one cigar had been smoked, about 25 to 30 minutes. The compression was maintained and pulsations recorded for some time, 10 to 20 minutes, after smoking was discontinued. During the course of the record the blood pressure was taken at about 5-minute intervals by the auscultatory and graphic methods, thus giving a quantitative check on our continuous record. Both smokers and non-smokers served as subjects. Eleven experiments were conducted but the data of three of them, experiments 1, 2 and 4, are not presented because we failed to take graphic and auscultatory blood pressure determinations along with the continuous record; therefore no quantitative blood pressure figures can be given in these. However, we may state that the same changes in the amplitude of oscillations were shown in them as in those presented.

Results. In every case a rise in both systolic and diastolic pressures was obtained. This is manifested, *1*, by an increase in the amplitude of the oscillations from the jacket set above systolic pressure; *2*, by a decrease in amplitude of the oscillations from the jacket set below diastolic pressure, and *3*, by the findings of the auscultatory and graphic blood pressure determinations. There was also usually some nausea and sometimes weakness. These symptoms appeared after 25 to 30 minutes of smoking or 30 to 35 minutes of compression in the jackets, this being soon after the blood pressure began to rise. They appeared with equal frequency in smokers and non-smokers; in fact, the most severe reaction, with a marked late fall in blood pressure and almost complete collapse, was in a regular smoker (exper. 7).

Digitized by Google

Experiment 7. Subject H. L., smoker.

Time in minutes

0 Blood pressure 106/70, pulse rate 71. Compressing pressure in jacket, 113 mm. Hg. Average amplitude of oscillations, 4.2 mm.

21 Blood pressure 130/86, pulse rate 98, average amplitude of oscillations, 5 mm.

31 Blood pressure 130/92, pulse rate 94, slight vertigo, smoking stopped, blood pressure begins to fall.

42 Blood pressure 100/68, pulse rate 68, subject weak, pale, sweating.

46 Blood pressure 92/60, pulse rate 59, pulsation oscillations barely visible, subject weak and cold. Pressure in jacket released, blood pressure at this time 88/60. Immediately on release of compression subject feels better.

52 Blood pressure 110/70, subject feels normal.

Another type of reaction is shown in experiment 5.

Experiment 5. Subject W. B. H., non-smoker.

Time in minutes

0 Blood pressure 105/70, compression jackets set at 114 and 67 mm. Hg., average amplitude from hypersystolic jacket 2.6 mm., from hypodiastolic jacket 10.5 mm.

33 Blood pressure 128/90, average amplitude from hypersystolic jacket 4.2 mm., from hypodiastolic jacket 4.4 mm., subject begins to feel weak.[1]

38 Blood pressure 105/85, average amplitude from hypersytolic jacket 2.8 mm., from hypodiastolic jacket 5.6 mm., subject sick and weak. Smoking stopped, compression continued.

42 Blood pressure 110/92, average amplitude from hypersytolic jacket 2.6 mm., from hypodiastolic jacket 2.8 mm., subject almost collapsed. Decompressed.

57 Blood pressure 112/82, subject feels normal. Sections of the record of this experiment are shown in figure 1.

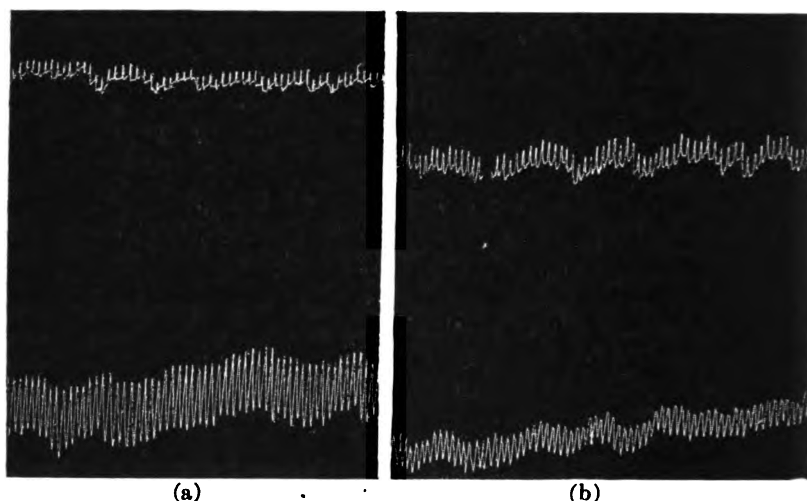
The result obtained in experiment 5 is in contrast with the preceding case where the blood pressure fell to subnormal during the compression. Why such severe symptoms should occur with a fall of blood pressure to its original level is not clear; they may probably be at least in part

[1] In these first experiments we were interested in the effect of smoking on blood pressure and our notes dealt with the time relations of the blood pressure changes to the smoking rather than to the compression, because at that time we did not realize that the compression was the factor bringing about the blood pressure changes. Since in all cases, however, the compression was applied about 5 to 8 minutes before smoking started, the period of compression can be ascertained from the latter; thus in the case at hand the blood pressure was 128/90 about 33 minutes after onset of compression, i.e., 26 minutes after beginning smoking.

Digitized by Google

explained by the effect of the smoking itself. This secondary fall of blood pressure while the compression is still being maintained is not constant in all the experiments. It occurred only in the smoking experiments and in by no means all of these. It is presumably due to a vasomotor fatigue, probably aggravated by the nicotine.

These results, a marked rise in blood pressure, followed in some cases by a fall and the accompanying unpleasant symptoms, are quite at variance with the findings of previous observers who have studied the effect of smoking on blood pressure (3), (4) and who find in non-smokers



(a) (b)

Fig. 1. Tracings of oscillations from jackets *a*, immediately after application of compression, and *b*, about 30 minutes later. Blood pressure at time of *a* was 105/70; at time of *b*, 128/90. Reversal of amplitudes of oscillations from hypersystolic and hypodiastolic jackets is well shown.

on smoking one or two cigars a rise of 10 to 20 mm. Hg. in systolic pressure, followed by a fall when the toxic symptoms usual in non-smokers appear, in moderate smokers a gradual rise of 8 to 10 mm. and in "excessive" smokers no change or a rise of 2 to 4 mm. It will be noted that our blood pressure changes are much greater than these, furthermore they occur in habitual smokers to the same extent as in non-smokers. The data of a typical experiment with a habitual smoker are given in table 1.

A control experiment was now run, i.e., compression applied to the subject's hands and forearms while he was not smoking.

Digitized by Google

Control 5. Subject K. A. M., non-smoker.

Time in minutes

0 Blood pressure 110/72, average amplitude from hypersystolic jacket 4.1 mm., from hypodiastolic jacket 3.4 mm., pulse rate 74. Compressing pressure in jackets 116 and 67 mm. Hg.

28 Blood pressure 120/78, average hypersystolic amplitude 4.5 mm., average hypodiastolic amplitude 3.1 mm., pulse rate 68.

35 Blood pressure 140/90, average hypersystolic amplitude 9.6 mm., hypodiastolic amplitude 3.1 mm., pulse rate 62.

40 Blood pressure 144/90, average hypersystolic amplitude 11.6 mm., hypodiastolic amplitude 3.2 mm., pulse rate 59.[3] Now decompressed. No markedly unpleasant symptoms, as with a combination of compression plus smoking. There was, however, some sensation of heat, throbbing in the head and some sweating.

40½ Blood pressure 135/76.

41 Blood pressure 130/76.

43 Blood pressure 120/72.

46 112/70.

49 112/70. Amplitude of oscillations could of course not be followed after decompression.

It thus became obvious that some factor other than the smoking was influencing the blood pressure. Accordingly this continuous graphic method of following blood pressure was dispensed with and the effect of blood pressure on smoking was followed by making blood pressure determinations at frequent intervals by the auscultatory and graphic methods, using the Erlanger instrument. The results of these observations have no further concern with this paper, except that it may be stated here that no significant blood pressure changes were found to result from smoking.

Leaving aside, then, the question of the effect of smoking on blood pressure, and passing on to a consideration of the effects of the compression, further controls without smoking were run, using the apparatus diagrammed in figure 2. Here only one jacket was used and oscillations from it were not recorded, the jacket serving merely as a compression

[3] It is of course recognized that the pressure in the hypersystolic jacket is no longer hypersystolic after such a rise in systolic blood pressure, but the changes in the amplitude of oscillations occur with the pressure in the cuff or jacket in the neighborhood of systolic; in case of such a rise as the one under consideration the increased pulse volume also contributes to the amplitude of oscillations; but it may be noted that the decrease in the amplitude of oscillations from the hypodiastolic jacket is occurring in spite of the tendency of the increased pulse volume to increase the amplitude.

Digitized by Google

chamber. The pressure in the jacket was set well above systolic and was recorded by a writing lever on the manometer float, *A*. The manometer, *B*, on the Erlanger sphygmomanometer was also made to record on the kymograph. Thus, after obtaining zero points, one could apply the pressure in the sphygmomanometer cuff and carry out graphic determinations by the continuous decompression method without watching to get the manometer reading; one can go back and measure the records at leisure. Furthermore, the subjective error due to reaction time is eliminated. Auscultatory blood pressure determinations were

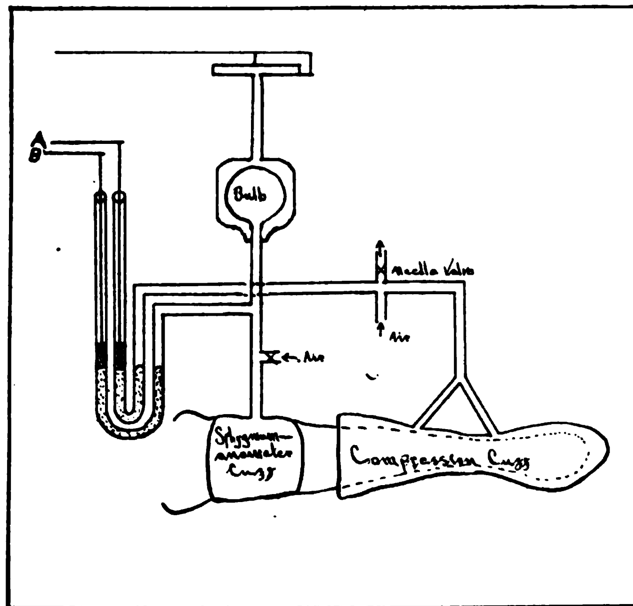


Fig. 2. Diagram of apparatus used in control experiments.

made on the opposite arm. The arrangement will be apparent on examination of the diagram of apparatus, figure 2, and of a typical record reproduced as figure 3. The results of one such control, sections of the record of which are shown in figure 3, may be given in some detail here.

Subject K. A. M. Initial blood pressure 114/75 by auscultatory, 113/76 by graphic. In every case the auscultatory figure is the average of two consecutive determinations which, except during the period of blood pressure waves, always checked within 2 mm. The corresponding graphic determination was made as

Digitized by Google

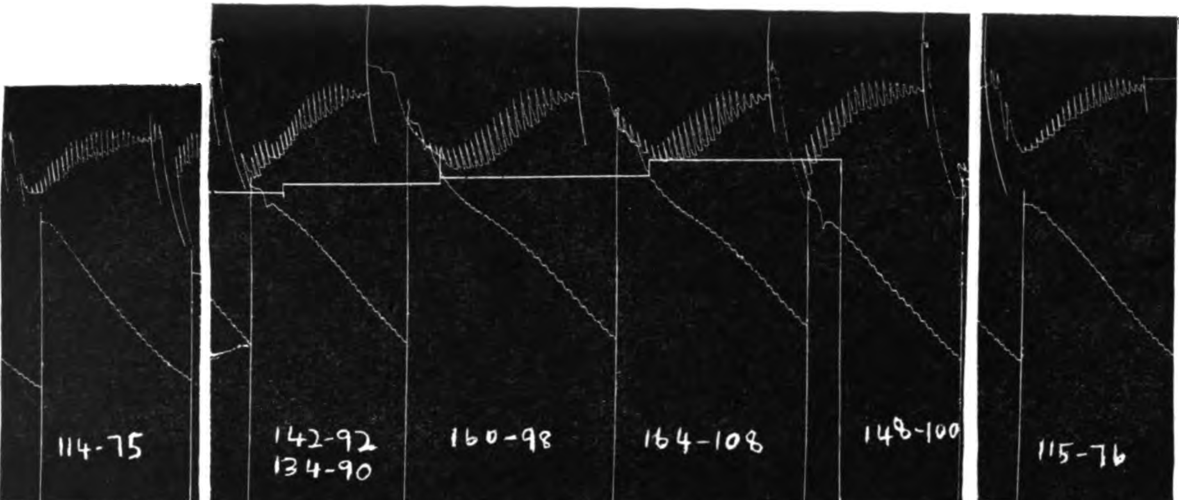


Fig. 3. Sections of record made in control 1. First section, normal, before compression. Application of compression in sphygmomanometer cuff records as vertical line, decompression as an oblique falling line. Actual blood pressure values by the graphic method can be measured by laying off the horizontal distance from the arc on tracing of oscillations to the oscillations presenting the systolic and diastolic criteria, respectively, then finding the level at which the falling pressure line is this horizontal distance from the vertical line. The height of this level above the base line, multiplied by four, since figure is reduced one-half, gives the blood pressure. Corresponding determinations by auscultatory method are written in the figure. In the second and fourth sections blood pressure waves are seen; e.g., at beginning of tracing the systolic pressure is above the cuff pressure, in a few pulses it falls below cuff pressure although the latter has itself fallen in the meantime; as the cuff pressure continues to fall it soon falls again below the systolic. The compressing pressure in the jacket is recorded as the horizontal line just beneath the oscillation tracings. It is seen to be released in section five. The last section shows a tracing made after blood pressure had returned to normal.

Digitized by Google

soon as possible, within a few seconds, after the auscultatory, and the auscultatory figure was written on the record under its corresponsing graphic determination as soon as it was taken. Compression of 150 mm. Hg. applied at 3:50 p.m. This compression was kept practically constant during most of the experiment, slight fluctuations due to variations in source of air pressure occurring, but pressure was always within a few millimeters of 150 and even the slight variations

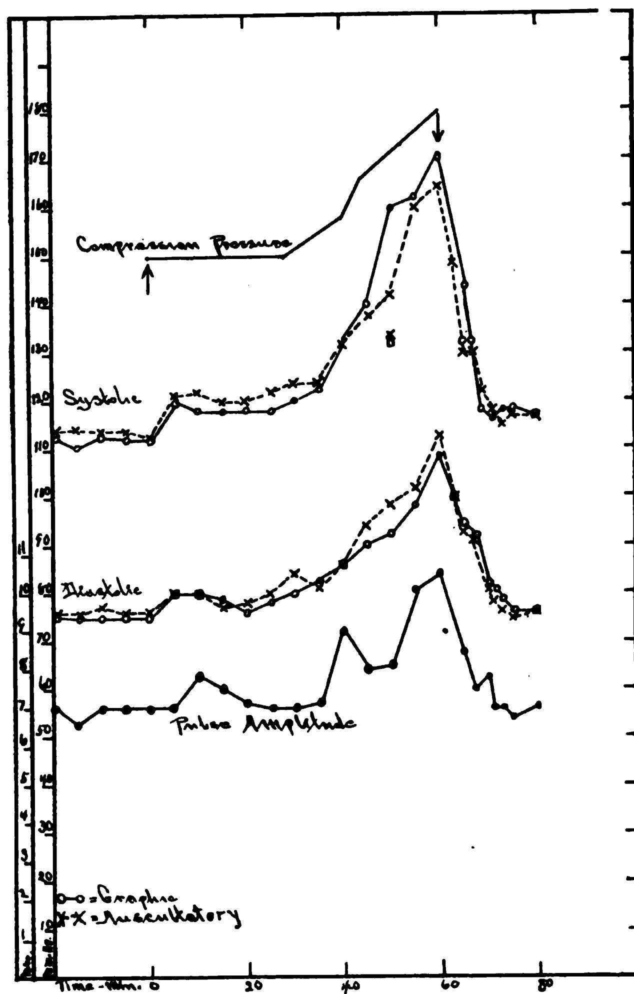


Fig. 4. Left vertical column of figures designates millimeters amplitude of oscillations in sphygmomanometer tracings. Right vertical column designates millimeters Hg. of blood pressure and compressing pressure.

Digitized by Google

were of brief duration. Toward the end of the experiment, as the blood pressure rose, the compressing pressure was gradually raised so as to exceed it slightly and was 180 mm. Hg. at the close of the experiment. Immediately upon application of compression blood pressure rose to 122/80 auscultatory, 120/80 graphic. At 4:16 blood pressure 124/80 auscultatory, 120/84 graphic. Shortly after this waves of blood pressure appeared. At 4:40 blood pressure was 136/90 auscultatory, 140/95 graphic. From this time on, at least until the highest pressures were established, there were marked waves of blood pressure; consecutive auscultatory determinations made at as short an interval as possible might show a difference of 20 mm. in the systolic reading. The same phenomenon can be seen in sections 2 and 4 of figure 3, which is made from the record of this experiment, the change in form in these being already present at the highest pressure shown, then disappearing and reappearing several pulses later on in the record. At 4:45 blood pressure was 160/98 auscultatory, 162/102 graphic. At this time subject complained of feeling hot, had a severe headache and was sweating profusely. Shortly after this the blood pressure was 164/108 auscultatory, 170/112 graphic. The arm was then decompressed. Three minutes later blood pressure was 148/100, auscultatory and graphic; 5 minutes later 130/94 auscultatory, 132/92 to 144?/92 graphic. Thirteen minutes after decompression blood pressure was 115/76 auscultatory and 118/79 graphic, subject felt normal. These results, as well as the amplitude of oscillations from the Erlanger instrument, are seen in graphic form in figure 4 and are tabulated in table 2. It will be seen that the amplitude of oscillations increases with the arterial pressure.

The symptoms in the control are not so severe as in the smoking experiments, although the blood pressure in some of the controls was run up higher than in some of the smoking experiments. The absence of nausea in the controls is the most striking difference. Undoubtedly the action of the tobacco superimposed upon the greatly disturbed circulatory condition is a factor in the production of symptoms but it is evident that the circulatory changes alone can produce definite symptoms. It will be noted that the rise in blood pressure begins earlier, within 20 to 25 minutes after compression starts, in the smoking experiments than in the controls, 35 to 45 minutes.

Tables 1 and 2 give the full data of one smoking and one control experiment, respectively. It is found impracticable to tabulate all the data of all the experiments. Tables 3 and 4 have therefore been constructed which give a summary of the data of all the eight smoking and five control experiments, respectively.[3]

Further investigation of the factor or factors bringing about the marked rise in blood pressure just noted seemed desirable. It could be

[3] N. S. and S. in tables 3 and 4, refer to non-smokers and smokers respectively. G and A, after blood pressure figures, refer to graphic and auscultatory methods, respectively.

Digitized by Google

ascribed neither to the tobacco nor to the sucking action of the smoking, as both of these were eliminated as factors in the controls. The explanation which first suggested itself was that the rise in blood pressure was a

TABLE 1

Data of a smoking experiment, no. 9. Habitual smoker.

	TIME	COMPRESSING PRESSURE IN "HYPERSYSTOLIC" AND "HYPODIASTOLIC" JACKETS	BLOOD PRESSURE, GRAPHIC AND AUSCULTATORY	MAXIMUM, MINIMUM AND AVERAGE OSCILLATIONS FROM "HYPERSYSTOLIC" JACKET	MAXIMUM, MINIMUM AND AVERAGE OSCILLATIONS FROM "HYPODIASTOLIC" JACKET	PULSE RATE PER MINUTE
	minutes	*mm. Hg.*	*mm. Hg.*	*mm.*	*mm.*	
Before compression			105–70			
			105–70			
After application of compression	5	113–67	105–70 G	3.1	4.8	65
			105–70 A	1.8	3.0	
				2.5	3.8	
	10	113–67	107–72 A	3.6	3.3	73
				1.6	2.3	
				2.5	3.1	
	24	113–67	120–80 G	8.5	3.8	71
				4.2	2.8	
				6.0	3.2	
	35	113–67	145–95 A	12.4	3.7	68
				6.8	2.4	
				9.2	2.8	
	41	113–67	142–92 G	14.5	3.4	
				9.4	2.4	68
				12.2	2.8	
After release of compression	1	137–85 G				
	6	120–78 G				
	9	112–74 G				
	14	110–78 G				

response to the local ischemia produced by the compression exerted on the arm in the jackets. It might be thought of as a compensatory effort to force blood into the tissues rendered anemic by the compression. Such a response is of course well known in the case of increased intra-

Digitized by Google

TABLE 2

Data of control 1. Non-smoker

	TIME	COMPRESSION PRESSURE	BLOOD PRESSURE, GRAPHIC AND AUSCULTATORY	AMPLITUDE OF MAXIMUM OSCILLATION
	minutes	*mm. Hg.*	*mm. Hg.*	*mm.*
Before compression			113–76 G 114–75 A	7.0
			111–76 G 115–75 A	6.5
			113–78 G 114–75 A	7.0
			112–76 G 114–75 A	7.0
			113–77 G 112–75 A	7.0
After application of compression	2	150	120–80 G 122–80 A	7.0
	6	150	118–80 G 122–80 A	7.8
	10	150	118–76 G 120–78 A	7.5
	15	150	118–78 G 120–76 A	7.2
	21	150	118–80 G 122–78 A	7.0
	26	150	120–84 G 124–80 A	7.0
	32	150	122–81 G 124–82 A	7.2
	40	150	132–86 G 132–85 A	9.0
	50	150	140–95 G 136–90 A	8.0

Digitized by Google

TABLE 2—*Concluded*

	TIME	COMPRESSION PRESSURE	BLOOD PRESSURE, GRAPHIC AND AUSCULTATORY	AMPLITUDE OF MAXIMUM OSCILLATION
	minutes	*mm. Hg.*	*mm. Hg.*	*mm.*
After application of compression			160–100 G	8.2
			134–100 G	
	52	150	142–92 A	
			134–90 A	
	55	170	162–102 G	10.2
			160–98 A	
	60	180	170–112 G	10.5
			164–108 A	
After release of compression	3		148–100 G	8.5
			148–100 A	
	5		132–92 G	7.5
			144?–92 G	
			130–94 A	
	7		132–90 G	7.8
			130–92 A	
	9		118–83 G	6.8
	9		122–84 A	
	11		116–82 G	6.8
			118–78 A	
	13		118–79 G	6.5
			115–76 A	
	15		118–76 G	6.5
			116–75 A	
	20		116–76 G	6.7
			116–76 A	

cranial pressure but so far as we knew at that time no such observations had ever been made upon extracranial tissue. Moreover, the conditions in the two cases are not strictly comparable, since in the case of increased intracranial pressure the vasoconstrictor center itself is pre-

Digitized by Google

TABLE 3

Summary of data of smoking experiments

EXPERIMENT NUMBER	INITIAL BLOOD PRESSURE	PRESSURE IN HYPERSYSTOLIC JACKET	HIGHEST BLOOD PRESSURE ATTAINED	TIME, AFTER COMPRESSION, OF APPEARANCE OF RISE OF BLOOD PRESSURE
	mm. Hg.	*mm. Hg.*	*mm. Hg.*	*minutes*
3 NS	108–74 A	118	122–84 A	17
5 NS	105–70 G 107–72 A	114	128–90 G 128–90 A	18
6 S	98–70	108	118–84	30
7 S	106–70	113	130–92	15
8 S	105–70	113	116–85	28
9 S	105–70 G 105–70 A	113	145–95 A	20
10 S	108–80 G	116	118–98	34
11 S	104–62 G 106–62 A	112	138–80	24

TABLE 4

Summary of data of control experiments

EXPERIMENT NUMBER	INITIAL BLOOD PRESSURE	COMPRESSING PRESSURE	HIGHEST BLOOD PRESSURE ATTAINED	TIME, AFTER COMPRESSION, OF APPEARANCE OF RISE OF BLOOD PRESSURE
	mm. Hg.	*mm. Hg.*	*mm. Hg.*	*minutes*
1 NS	112–76 G 114–75 A	150	170–112 G	40
2 S	108–70 G	135	134–80 G	?
3 NS	112–72 G	135	144–85 G	40
4 NS	112–74 A	158	144–100 A	44
5 NS	110–72 G 112–72 A	116	144–90 A	23

Digitized by Google

sumably rendered ischemic and thereby stimulated, while in the case at hand the local ischemia must bring about the rise in blood pressure either reflexly, chemically or in some other way than by direct stimulation of the vasomotor center such as obtains in the case of increased intracranial pressure.

Accordingly the pressure was set in the jacket at different levels in order to determine at what level a rise in blood pressure would be produced. It was found that there was practically no change in blood pressure until the pressure in the jacket was raised above the systolic pressure. Also, there was not an exact correspondence between the height of the pressure in the jacket and the extent to which the blood pressure would be raised, but in every case except two, controls 1 and 4, the systolic blood pressure rose to a height greater than that in the jacket, up to 165 mm. Hg. In one of these exceptions, control 4, the pressure was rising rapidly when the compression was released and there is no reason to believe that it would not have exceeded the compressing pressure if the latter had been kept on a short time longer. In the other exception, control 1, the blood pressure was not given a chance to rise above the compressing pressure, for as the former rose the latter was purposely raised to keep it above the former. Thus at the close of the experiment conducted in this way the compressing pressure was 180 mm. Hg., the systolic blood pressure 170 mm. Hg. This failure of exact correspondence may be due to variations in the relation of arteries to the bony framework in different individuals, requiring greater excess above systolic to render one person's forearm ischemic than is required for another. The fact that under a compression 10 mm. Hg. above systolic the systolic pressure might rise 30 to 40 mm. indicates, as is of course well known, that the circulation is still seriously interfered with even though the compressing pressure may no longer exceed the systolic pressure. It will also be noted that in one and the same individual the time required for the development of the rise of pressure varies from 25 to 45 minutes.

An effort was next made to determine the mechanism acting to produce this rise of blood pressure. The pressure in one jacket was set 27 mm. above systolic while the other jacket was used as a plethysmograph. If the rise in blood pressure were due to a vasoconstriction we should expect a diminution in the volume of the plethysmographed arm coincident with the rise in blood pressure. It was found, however, that the volume of the plethysmographed arm *increased* during the rise in blood pressure. This increase was maintained during the period of

Digitized by Google

heightened blood pressure. The arm volume receded rapidly when the blood pressure fell on release of the pressure on the other arm. Some of the details of this experiment are here given.

Plethysmograph tracing with compression.

Time in minutes	
0	Blood pressure 108/70. Pressure in compression jacket 135 mm. During first half-hour no significant change in plethysmograph tracing or in blood pressure.
30	Blood pressure 116/74.
35	Blood pressure 122/78. Plethysmograph curve starts up rather rapidly, steady upward trend.
40	Blood pressure 134/76. Plethysmograph curve going slowly but steadily upward. Arm now decompressed. Plethysmograph tracing at once assumes a consistent downward trend, blood pressure at same time falling.
44	Blood pressure 116/74, plethysmograph tracing coming down steadily.
48	Blood pressure 114/76, plethysmograph tracing coming down consistently with a gradual retardation.

It seemed, then, that we had shown that the blood pressure rises to overcome a local ischemia produced by compression of extracranial tissue and that this rise is not accompanied by vasoconstriction in a plethysmographed arm. Possibilities for further investigation on the human subject as to the nature of this response seemed practically exhausted. Accordingly we turned to animal experiments for further light. Naturally, one of the first questions which presented itself was whether or not we had in our observations a clue to the explanation of one type of clinical hypertension. We might conceive of scar tissue in the renal capsule contracting and subjecting the kidney to a pressure great enough to interfere with renal circulation, the response being a rise in blood pressure. Or local areas of kidney tissue might be thus affected by interstitial scarring, or an analogous condition might obtain in other organs. Such a conception is of course not original but so far as we knew no one had subjected this conception to an experimental test.

A compression chamber was therefore devised by which pressure could be applied to the kidney without occluding the renal vessels or ureter. It consisted of two airtight bladders, one to be placed on each side of the kidney, and a brass case to surround these. This was made in two halves which could be quickly taken apart. The vessels and ureter came out through openings cut into the approximating edges of the halves. The case itself was not airtight, the air pressure being

Digitized by Google

applied inside the bladders, the case acting merely as a guard for the bladders. Precautions were taken in applying the chamber not to injure the renal nerve supply passing through the pelvis.

In the first experiment a rabbit was used. Chloral and urethane were administered by stomach tube one half-hour before experiment started and were followed by light ether anesthesia. Cannula in right carotid. Compression chamber placed on left kidney through lumbar incision. Initial blood pressure 50 mm. Hg. Pressure to which kidney was subjected and blood pressure were recorded on kymograph, same base line being used, so that relation of pressure on kidney to blood pressure could be seen at a glance. Animal in only fair condition. After several minutes of constant blood pressure a pressure of 60 mm. Hg., about 10 mm. above blood pressure, was put on the kidney. No change in blood pressure resulted. This was repeated several times, the pressure being raised as high as 100 mm. Hg. and the duration of the pressure varying from 30 seconds to 5 minutes. In no case was any significant change in blood pressure observed. At the conclusion of the experiment the pressure was raised to 70 mm. Hg. and the renal vessels occluded by a clip. Kidney then decompressed and chamber removed. Kidney seen to be blanched. Clip then removed from vessels, when kidney immediately became red and was distinctly seen to increase in volume; at the same time slight hemorrhage started from the small capsular vessels which had been torn in removing the pericapsular fat while putting on the compression chamber. This seemed to be good evidence that the kidney had really been rendered anemic by compression.

The experiment was repeated with a dog, using ether anesthesia. The same result, i.e., no significant change in blood pressure, was obtained. Another experiment with a decerebrate cat as subject also was negative.

It seemed possible that the rise in blood pressure obtained in man might be due to the discomfort produced by the compression. In order to investigate this point a human subject, K. A. M., was given "twilight," the morphine-scopolamine anesthesia regularly used on the obstetrical service in Barnes Hospital. An initial subcutaneous injection of morphine, grain $\frac{1}{6}$ and scopolamine, $\frac{1}{133}$, followed in 45 minutes and 90 minutes by scopolamine, grain $\frac{1}{133}$, was given. At the end of 90 minutes the subject was in the peculiar state which has been clearly described by H. Schwarz (5). In the case at hand the subject was quite unconscious although he stirred restlessly at times. At the conclusion of the experiment he had no recollection of what had happened. He did not recover consciousness sufficiently to walk unassisted until 3 hours after the conclusion of the experiment. The results of this experiment were the same as those with the conscious subjects, i.e., a marked rise of blood pressure, the systolic rising from 115 to 155 and the diastolic from 75 to 85 mm. The pressure in the jacket was set at 130 mm. Hg., 15 mm. above the initial systolic. Owing to the inability of the

Digitized by Google

subject to coöperate it was found impracticable to record the pulsations but blood pressure determinations were made at frequent intervals with both graphic and auscultatory methods. The rise in blood pressure was initiated 32 minutes after application of pressure and was maintained until release of pressure, 45 minutes after its application. Blood pressure fell rapidly after release of the pressure in the jacket and in 6 minutes had reached its initial level. Twilight alone produces no significant blood pressure changes.

Discussion. It seems apparent that a rise in blood pressure occurs in the human subject beginning 25 to 45 minutes after, and reaching its maximum 35 to 60 minutes after a pressure exceeding systolic blood pressure is applied to the forearm and hand. This rise apparently is not due to a somatic vasoconstriction. It has also been seen that this rise is not dependent upon any subjective reactions to the conditions of the experiment. On the other hand, we have failed to obtain any significant changes in blood pressure as a result of briefer periods of kidney compression in either anesthetized or decerebrate animals. Before attempting to reconcile these findings let us consider briefly the possible significance of the unquestionably positive findings in the human subject.

First, it is apparent that the mechanism producing this response is not mechanical forcing of the blood from the arms into the restricted circulatory bed. The long latent period eliminates this explanation. It also shows that this is not an ordinary pressor reflex, which is immediate in its response. Just what the mechanism is is not clear. We might conceive that owing to some metabolic disturbance in the ischemic tissues they elaborate a toxic pressor substance which slowly accumulates until it is present in concentration sufficient to produce physiological effects. This substance must then act locally on the peripheral nerves, since it is excluded from the circulation. Its action cannot be that of adrenalin for a vasoconstriction of the forearms alone could not produce such a marked rise in blood pressure; furthermore a vasoconstriction in the area of production of this hypothetical substance could not raise the blood pressure, since this area is already excluded from the circulation by the external pressure. We may present then, the conjecture that the hypothetical toxic substance acts upon the afferent fibers, producing an increasing pressor reflex as it accumulates. When the circulation is restored to the forearm this substance may be quickly oxidized, permitting the blood pressure to fall to normal. In the instances in which, while smoking, the blood pressure fell to a subnormal level and

Digitized by Google

collapse seemed imminent while the pressure was still being exerted upon the arm, it may be supposed that vasomotor fatigue supervened and perhaps was aggravated by the nicotine.

The region of vasoconstriction is probably splanchnic. The plethysmograph observation referred to above, in which it was seen that the volume of one forearm increased while the other was under compression and the blood pressure was rising, shows that the vasoconstriction does not include the opposite arm and presumably, therefore, none of the so-called somatic area, although it is recognized that the latter presumption must be made with considerable reservation. It is highly probable, then, that by far the most powerful vasomotor influence is exerted upon the splanchnic area and that the increase in volume of the opposite arm is purely passive.

In regard to our failure to get positive results in the animal experiments, it is apparent in the first place that the two sets of experiments are not analogous in that the time of compression in the animal experiments was not as long as the time required for the rise in blood pressure to make itself manifest in the human experiments. Longer periods were not used because it became evident that a compression of the kidney lasting 30 minutes would injure it so severely that even if a rise in pressure did occur we would not be justified in assuming that it was due to the same factor operating in our human experiments and also because we would have no return to normal conditions. We therefore felt that it was not practicable to carry out on an animal's kidney the same procedure carried out on a man's forearm.

We had conducted two experiments on rabbits, with negative results, when we found that Alwens (6) had preceded us in this method. Alwens found that on subjecting both kidneys of a cat to a pressure above systolic blood pressure there was an *immediate* rise in blood pressure of a few mm. (5 to 10) and an immediate fall in blood pressure on release of compression pressure. The latent period was imperceptible. He finds this occurring even after the splanchnic nerves have been cut, and that it disappears on ligation of the renal arteries. He does not ascribe any importance to this slight rise as an explanation of clinical hypertension, admitting that it is certainly due to a direct transmission of the pressure exerted upon the kidneys out through the renal arteries to the aortic stream. The reason for our failure to obtain a similar rise on kidney compression is due to the fact that we compressed only one kidney, the mechanical effect of this not being great enough to produce an appreciable change. In a case where Alwens compressed only one

Digitized by Google

kidney he obtained a rise in blood pressure of 4 mm. Hg. Such a rise cannot be regarded as of any significance; in fact, we obtained changes of this magnitude without attributing any significance to them. Alwens failed to obtain blood pressure changes on compression of the hind limb of dogs for brief periods. This he explained as being due to the indirectness of the transmission of the pressure to the aortic stream. It is obvious that the phenomenon with which we are dealing in our 40 minute periods of compression of the forearm is quite different from that dealt with by Alwens. As we have stated, we found that practical difficulties rendered it impossible to subject the kidney to a compression of the same duration as was practicable with the forearm. We may, then, reject both Alwen's and our own animal experiments as having no bearing on the phenomenon under consideration.

The idea that a rise in blood pressure might be a compensatory response to a local ischemia produced by a contraction of scar tissue, as in chronic interstitial nephritis, is, of course, an old one. So far as we can discover, however, Alwens was the first to attempt to reproduce this condition experimentally, and his attempt was unsuccessful. Our positive results were obtained by subjecting tissues to a much longer period of compression; and it is this difference in the method employed that undoubtedly accounts for the difference in results of previous observers and of ourselves. Alwens assumed that the response would be similar to that of intracranial tissue to compression, i.e., almost immediate, and his work was discontinued upon failure to get this immediate response. So far as we are aware ours is the first demonstration of a response by an increase in blood pressure to local ischemia produced by compression of extracranial tissue. We believe that there is a strong probability of a connection between these results and the mechanism of certain types of clinical hypertension. We feel that it is impossible to produce experimentally in the kidneys by compression conditions similar enough to those obtaining in kidney disease to subject our views to a fair test on kidney tissue, but we believe that we have succeeded in producing an analogous condition in the arm tissue, with strikingly positive findings.

SUMMARY

Compression of the forearm great enough to produce a local ischemia produces a rise in systolic and diastolic arterial pressures, the systolic pressure tending to rise above the compressing pressure.

Digitized by Google

The rise in pressure does not manifest itself until the compression has lasted 15 to 45 minutes, and then is usually maintained as long as the compression is maintained, at least up to 60 minutes. It appears earlier with smoking than without. Headache, sweating and a sensation of heat usually accompany the rise in pressure; in smoking nausea also is usually present, even in the case of habitual smokers.

Marked waves of blood pressure have been observed to initiate the rise. Whether or not this is a constant phenomenon is not certain.

The arterial pressure rapidly returns to its initial level on release of compression. In 2 cases the blood pressure fell to or below its initial level before decompression. This was presumably due to vasomotor fatigue and was accompanied by symptoms of collapse.

The rise in pressure is probably due to splanchnic vasoconstriction. It is accompanied by an increase in volume of the opposite arm, which is probably passive.

No significant change in blood pressure resulted on brief compression of the kidney in the anesthetized rabbit and dog, or in the decerebrate cat.

The suggestion is made that these findings may have some bearing on the etiology of clinical hypertension.

The authors wish to thank Doctor Erlanger for numerous helpful suggestions made during the course of the work.

BIBLIOGRAPHY

(1) Erlanger and Festerling: Journ. Exper. Med., 1912, xv, 370.
(2) Foley, Coblents and Snyder: This Journal, 1916, xl, 555.
(3) Lee: Quart. Journ. Exper. Physiol., 1908, 1, 335.
(4) Fisher and Berry: The physical effects of smoking.
(5) Schwarz: Amer. Journ. Obstet., 1919, lxxix, 46.
(6) Alwens: Deutsch. Arch. f. klin. Med., 1910, xcviii, 137.

Digitized by Google

THE SIGNIFICANCE OF HYDREMIA IN THE SECRETION OF URINE

HARRY BAKWIN

From the Pediatric Division of the New York Nursery and Child's Hospital, and the Department of Pediatrics, Cornell University Medical College, Dr. L. C. Schroeder, Acting Director

Received for publication January 3, 1922

It is generally assumed by adherents of the modern theory of urinary secretion (1) that the diuresis following the drinking of large quantities of water is due to a dilution of the blood. The decrease of the osmotic pressure of the blood plasma incident to the hydremia, it is believed, favors filtration through the renal capsule.

There is, however, little direct experimental evidence to support this assumption. Engel and Scharl (2) studied the blood concentration and urine output after 1300 to 1400 cc. of fluid by mouth. Their patients showed diuresis but no diminution in the refractive index of the blood. In some of their experiments the blood became more concentrated during the height of the diuresis. MacCallum and Benson (3) produced profuse diuresis by drinking 2700 cc. of water. They also were unable to detect a diminution in the concentration of the blood with the hemocytometer. Haldane and Priestley (4) and Priestley (5) induced diuresis by drinking large quantities of water and saline. They found that the hemoglobin remained unaltered after water drinking but a slight reduction in the electrical conductivity of the serum occurred. After saline the hemoglobin was slightly diminished and the electrical conductivity of the serum increased. The diuresis after saline was less intense but more prolonged than after water drinking. They concluded that the diuresis following the drinking of fluids was not due to a dilution of the blood.

Ginsberg (6) studied the relation between hydremia and diuresis by comparing the amounts of urine secreted after water and saline drinking and after intravenous injections of the same substances. He found that the diuresis produced by giving the fluids intravenously was much less marked than that produced by giving smaller amounts by

Digitized by Google

mouth. He concluded that hydremia was not the important factor in causing water and saline diuresis. Cow (7) confirmed Ginsberg's findings and isolated a diuretic substance from the duodenal wall. He believed that when fluids were drunk this diuretic substance was washed into the circulation and produced the diuresis.

During the course of some studies on the fluid treatment of infants with diarrhea, data on the relation between blood concentration and urine output were obtained. These seem of sufficient importance to be reported here.

The method of study was as follows: Normal male infants were given 30 to 35 cc. of fluid per kilogram of body weight and the urine collected for four hours. Blood samples for refractometric determination were taken immediately before and at frequent intervals after the fluid was drunk. The water was sweetened with a little saccharine. Saline was given by gavage.

The serum concentration was determined with the Abbe refractometer. To prevent temperature variations, all the specimens from a single experiment were read simultaneously. Readings were made at temperature of 19 to 21° and distilled water was read each time. Blood was collected in Wright capsules. These were sealed and kept on ice.

The method of Robertson (8) was used to determine the refractive index of the non-protein substances of serum. The method consists of the precipitation of the serum proteins with weak acetic acid and heat. The refractive index of the acetic acid filtrate and of weak acetic acid are determined. From the difference between these two readings the refractive index of the non-proteins is calculated. We found that in the serum of well infants this ranged between 0.0016 and 0.0024. It was generally around 0.0020. We consequently used this latter figure in our calculations. 0.0020 was added to the reading for the refractive index of water, the sum was subtracted from the reading for whole serum. This gives the refractive index of the serum proteins. By dividing this figure by 0.00172, the value given by Reiss (9) for the refractive index of 1 per cent of protein, the percentage of protein is obtained. The method of calculating the serum protein percentage from the refractive index of serum may be represented as follows:

S = Refractive index of serum.
W = Refractive index of water read at the same temperature as the serum.
0.0020 = Refractive index of the non-protein substances of serum.
0.00172 = Refractive index of 1 per cent of serum protein (Reiss).

Digitized by Google

P = Refractive index of the serum proteins.
P = S − (W + 0.0020).

$$\frac{P}{0.00172} = \text{Percentage of serum protein.}$$

Urine was collected by means of a curved glass tube, one end of which was drawn over the infant's penis and attached to the pubis and perineum with adhesive plaster. The other end was attached by means of glass and rubber tubing to a bottle which hung at the side of the bed. An electric buzzer announced the time of each voiding. The head of the bed was raised and the infant's legs gently restrained. This apparatus has been in use at this hospital for some time and has been found a great convenience in the complete collection of urine.

TABLE 1
Showing the results in water drinking experiments

EXPERIMENT NUMBER	SERUM PROTEIN PER CENT BEFORE WATER DRINKING	LOWEST POINT TO WHICH SERUM PROTEINS FELL AFTER WATER DRINKING	AMOUNT OF FLUID DRUNK	AMOUNT OF URINE VOIDED	PERCENTAGE OF INGESTED WATER EXCRETED IN FOUR HOURS
			cc.	*cc.*	
1	7.37	6.68	210	133	60
2	6.74	5.81	180	55	30
3	7.64	6.91	120	35	29
4	8.24	7.61	180	13	7
5	7.26	6.74	210	69	33
6	6.93	6.33	135	52	38
7	7.84	6.91	135	70	51
8	6.68	5.75	120	46	38
9	8.43	6.80	105	16	15
10	7.43	6.57	105	88	83
11	7.09	6.91	135	82	61
12	8.02	7.84	210	220	105

Results. The results of 12 experiments on 10 infants after water drinking are shown in table 1. (See also protocols at the end of the paper.) In 10 of the 12 experiments there was a distinct hydremia as indicated by a diminution in the refractive index of the blood serum. This started immediately after the water was drunk, reached its height in from thirty minutes to two hours and then gradually disappeared. The maximum dilution usually amounted to about 0.5 to 0.9 per cent of protein but in one instance the decrease in serum protein amounted to 1.63 per cent (exper. 9). In experiments 11 and 12 there was no significant change in the blood concentration.

Digitized by Google

In but 1 of the 12 cases was there excessive secretion of urine. In the others the amount of urine voided during the four-hour period following the water drinking varied from 7 to 83 per cent of the water drunk. Usually about 35 per cent was excreted. In experiment 9 where the serum protein dropped from 8.43 per cent to 6.80 only 15 per cent of the water drunk was excreted in the four-hour period. Baby E. T. (exper. 12), whose blood showed no significant diminution in concentration after the water, voided 105 per cent of the water drunk.

Seven experiments were conducted after saline gavage (table 2). In two instances no significant dilution of the blood occurred. The remaining five showed distinct hydremia. As after water drinking, the blood dilution started soon after the saline had been given and reached its height from thirty minutes to two hours afterwards. In

TABLE 2

Showing results in saline gavage experiments

EXPERIMENT NUMBER	SERUM PROTEIN PER CENT BEFORE WATER DRINKING	LOWEST POINT TO WHICH SERUM PROTEINS FELL AFTER WATER DRINKING	AMOUNT OF SALINE GIVEN	AMOUNT OF URINE VOIDED	PERCENTAGE OF INGESTED SALINE EXCRETED IN FOUR HOURS
			cc.	*cc.*	
1	5.81	5.58	200	0	0
2	7.26	5.99	200	24	12
3	7.31	7.03	180	18	10
4	6.39	6.57	180	17	9
5	7.20	6.54	200	0	0
6	7.55	5.93	100	14	14
7	7.67	6.51	200	31	16

three instances the hydremia was preceded by a brief period in which the blood became concentrated. The blood concentration in 4 experiments did not return to its pre-fluid level in four hours (table 2, exper. 2, 5, 6, 7). The hydremia after saline gavage was more marked than after water drinking, amounting, in 4 of the 7 experiments, to over 1 per cent of protein.

Following the saline there was a distinct oliguria. The largest amount of urine secreted in four hours was 16 per cent of that given. In two instances (exper. 2 and 5) no urine was voided during the four-hour period. These results correspond with those of Ohlman (10), who similarly found a retention of fluid after saline drinking in infants.

In our series of experiments, then, hydremia without diuresis was produced. In general, in those instances where the blood dilution was

Digitized by Google

greatest the urine output was smallest. After the saline gavage where the serum often fell more then 1 per cent the oliguria was marked. It seems clear that the diminution in plasma protein concentration and the consequent decrease in the osmotic resistance of the plasma to filtration through the renal capsule are not sufficient to cause diuresis in well infants.

SUMMARY AND CONCLUSIONS

1. The concentration of the blood and the secretion of urine were studied in a group of well infants after water and saline drinking.
2. After water drinking a moderate hydremia occurred without diuresis.
3. After saline drinking there was a marked blood dilution and oliguria.
4. In infants, hydremia alone does not necessarily cause diuresis.

A few illustrative protocols are appended.

BIBLIOGRAPHY

(1) CUSHNY: The secretion of urine, London, 1917, 144.
(2) ENGEL AND SCHARL: Zeitschr. f. Klin. Med., 1906, lx, 225.
(3) MACCALLUM AND BENSON: Journ. Biol. Chem., 1909, vi, 87.
(4) HALDANE AND PRIESTLEY: Journ. Physiol., 1916, l, 296.
(5) PRIESTLEY: Journ. Physiol., 1916, l, 304.
(6) GINSBERG: Arch. f. exper. Path. u. Pharm., 1912, lxix, 381.
(7) COW: Arch. f. exper. Path. u. Pharm., 1912, lxix, 393.
(8) ROBERTSON: Journ. Biol. Chem., 1915, xxii, 233.
(9) REISS: Ergebn. d. inner. Med. u. Kinderheilk., 1910, x, 531.
(10) OHLMAN: Zeitschr. f. Kinderheilk., 1920, xxvi, 291.

WATER DRINKING EXPERIMENTS

Experiment 4. Hydremia with oliguria after water drinking

R. A. Age 6 months, weight 5.7 kgm., 180 cc. water

TIME	SERUM PROTEIN	AMOUNT OF URINE
	per cent	*cc.*
9:55 a.m.	8.24	
10:01–10:20 a.m.	Water drinking	
10:20 a.m.	7.84	
10:32 a.m.	7.73	
11:01 a.m.	7.61	
11:54 a.m.	7.61	
12:00 m.		9
1:00 p.m.	8.18	
1:40 p.m.		4
Total urine....................................		13

Digitized by Google

Experiment 9. Marked hydremia with oliguria after water drinking

H. S. Age 4 months, 4.6 kgm., 105 cc. of water

TIME	SERUM PROTEIN	AMOUNT OF URINE
	per cent	*cc.*
10:15 a.m.	8.43	
10:20–10:25 a.m.	Water drinking	
11:00 a.m.	7.61	
11:45 a.m.	6.96	
12:20 p.m.	6.80	
12:30 p.m.		16
1:45 p.m.	6.90	
Total urine..		16

Experiment 12. Diuresis without hydremia after water drinking

E. T. Age 8 months, 6.4 kgm., 210 cc. of water

TIME	SERUM PROTEIN	AMOUNT OF URINE
	per cent	*cc.*
10:10 a.m.	8.02	
10:14–10:20 a.m.	Water drinking	
10:20 a.m.		19
10:25 a.m.	8.19	
10:50 a.m.	7.84	30
11:00 a.m.		40
11:20 a.m.	8.24	
11:50 a.m.		57
12:00 m.	8.02	
1:07 p.m.		26
1:15 p.m.	7.84	
2:50 p.m.	8.02	
2:15 p.m.		48
Total urine..		220

Digitized by Google

SALINE GAVAGE EXPERIMENTS

Experiment 2. Marked hydremia and oliguria after saline gavage

Age 5 months, weight 4.7 kgm., 200 cc. of saline

TIME	SERUM PROTEIN	URINE
	per cent	*cc.*
10:05 a.m.	7.26	
10:11–10:13 a.m.	Saline gavage	
10:14 a.m.	7.61	
10:25 a.m.	5.99	
10:35 a.m.	6.51	
11:06 a.m.	6.68	
12:12 p.m.	6.57	
1:03 p.m.	6.74	
2:07 p.m.	6.74	
2:15 p.m.		24
Total urine....................................		24

Experiment 6. Marked hydremia and oliguria after saline. Palmer method used for the determination of hemoglobin

T. P. Age 3 months, 3.6 kgm., 100 cc. of saline

TIME	HEMOGLOBIN	SERUM PROTEIN	URINE
	gm. per 100 cc.	*per cent*	*cc.*
10:15 a.m.	15.0 gm.	7.55	
10:30 a.m.	Saline gavage		
11:00 a.m.	13.5 gm.	5.93	
11:30 a.m.	14.6 gm.	6.68	
12:15 p.m.	15.0 gm.	6.91	14
1:45 p.m.	14.1 gm.	6.57	
Total urine..			14

Digitized by Google

A STUDY OF THE REGENERATION OF THE AUTONOMIC FIBERS IN THE VAGUS NERVE OF THE SHEEP

STANLEY ROSS BURLAGE

From the Physiological Laboratory, Cornell Medical College, Ithaca, New York

Received for publication December 27, 1921

Although various incomplete summaries of the work done on the regeneration of the cardiac fibers in the vagus nerve have appeared at different times, it may not be amiss to give the actual conclusions reached together with the definite data from which these were drawn.

As early as 1878 Gluck (1), working with rabbits, was under the impression that he had established the certainty of a vicarious function in the cardiac fibers of the vagus in the case of one animal, within 10 days after cutting. He also found that in other instances manipulation of a severed and sutured vagus gave active swallowing movements some time later, "partiellen Restitution der Function." No definite time for recovery of function is stated in these cases. However it clearly antedates the work of Vanlair (2).

The latter found that when the vagus in a dog was cut and the ends sutured together, he obtained recovery of function in the recurrent laryngeal branch in 11 months. He was able to demonstrate no return of function in autonomic fibers.

Tuckett (3) first either cut or crushed the left vagus nerve in 4 rabbits and found that he got no regeneration of autonomic fibers, such that electrical stimulation of these nerves would cause a change in heart rate, in from 89 to 231 days. Later however (4) he reported that in the case of 3 rabbits in which he had either cut or crushed the left vagus, he was able to produce slowing of the heart by stimulation of the injured nerve in 2 of them after from 3 years, 8 days to 3 years, 38 days. This is the first authentic record of regeneration of the autonomic cardiac fibers which has come to the writer's attention.

In a dog, Stewart (5) found evidence of cardiac response to stimulation of the right vagus nerve, which had been cut 300 days before.

This work was repeated by Rogers (6) on 4 cats and 3 dogs. In the cats he found no regeneration in from 2 to 6 months and in 2 of the

Digitized by Google

dogs none in from 4 to 16 months. In the third dog however the right vagus had been cut and in 20 months the heart was definitely slowed by stimulation of this injured nerve.

Langley (7) cut the right vagus in a kitten and found no functional regeneration after 12 months.

In Schafer's laboratory the most extensive study of the subject has been carried on by several investigators. Tsukaguchi (8) severed the right vagus in 2 dogs and failed to get cardiac response upon stimulation 173 to 175 days later. Schafer and Feiss (9) cut the left vagus in 2 cats and stimulated the severed nerve 12 months, 9 days to 13 months later; cut the right vagus in 3 cats and examined 10 to 11 months later; severed the right vagus in 1 rabbit and investigated 74 days afterward; cut the right vagus in 1 dog and examined 11 months later. In no case was regeneration of cardiac fibers indicated.

Schafer (10) published the results of his own work in which the left vagus of a dog was cut and examined 1 year, 10½ months later. The right vagus was cut in 5 cats and investigated from 1 year, 5 months to 2 years, 50 days later. No evidence of functional recovery of the nerve was found in either the cats or the dog.

Up to date, all of the work upon regeneration of the autonomic fibers in the vagus has shown that the cardiac fibers in the right vagus of the dog may in some instances again become functional in from 300 days to 20 months, and that similar results may follow severing the left vagus of the rabbit in from 3 years, 8 days to 3 years, 38 days.

Experimental. In December, 1920, 5 sheep, 4 ewes and 1 ram were procured for observation. The normal heart and respiration rates were obtained by keeping the animals quietly standing in the corner of a room for 10 or 15 minutes. Then the heart rate was determined by auscultation. When all of the results were satisfactory, a unilateral vagotomy was performed upon each sheep.

In two ewes the left vago-sympathetic trunk was cut and in the remaining two ewes and the ram the right vago-sympathetic was severed. All nerves were cut at the level of about the middle of the thyroid gland.

In the sheep the cervical sympathetic is usually so closely bound to the vagus by connective tissue that, to avoid possible injury to both nerves, both were cut across instead of the vagus alone. After cutting, the ends of the trunks were approximated and held by a fine silk suture.

Before tying, however, the peripheral ends of the cut nerves were stimulated by induced current and the heart rate observed by placing the bell of a stethoscope over the apex region.

Digitized by Google

During the operation the sheep was placed upon its back and held. The field of incision was infiltrated with a 1 per cent solution of apothesine. It was found that about 7 cc. of this solution gave satisfactory local anesthesia.[1]

To avoid the death of the sheep by asphyxiation in case the cutting of the one inferior laryngeal nerve caused occlusion of the larynx, due to collapse of a thyreo-arytenoid ligament, trachea tubes were inserted in two of the ewes before the incision was sutured. These tubes were cleaned daily.

After 5 or 6 days, observations were again made upon the heart and respiration rate, with each animal quiet, as before the operation.

Three ewes and the ram died from 1 to 8 months after operation.

On December 8, 1921, 363 days after the right vago-sympathetic trunk had been cut and sutured, the remaining ewe was brought up for observation.

The sheep was anesthetised with ether, a tracheal cannula placed in position and ether alone was continued throughout the experiment.

A glass cannula was placed in the right common carotid artery and connected with a mercury manometer. Another cannula was placed in the right femoral artery and attached to a Hürthle manometer. A pneumograph was arranged to record abdominal respiration. The manometers and the recording tambour of the pneumograph were arranged to record on the same vertical line with a signal magnet and time recorder, on a long paper kymograph. It will be noted in the tracing that the signal magnet was accidentally displaced slightly to the right of the line of the other recording instruments.

Cohn (11) lead electrodes were placed on the right fore leg and left hind leg so that simultaneous electrocardiograms could be taken.

The right vago-sympathetic trunk was cut and the peripheral end repeatedly stimulated with varying strengths of tetanizing current. Then the left vagus trunk (exclusive of the sympathetic) was tied, cut and stimulated.

Discussion. Of the five sheep operated upon, four died. Three were old sheep and were used for this experiment in the hope that they might live to its completion. Their deaths were probably not due to the operation. Two were young healthy ewes and of these one died of pneumonia following lambing. The remaining ewe was the one investigated.

[1] Dr. Martin B. Tinker kindly advised on this method of anesthetising rather than a general anesthetic, in order to avoid pneumonia following the operation.

Digitized by Google

In a comparison of the respiration and heart rate while the sheep were standing quietly, it was found that in the four cases in which records had been kept the heart rate was about the same before and a week following the operation. The respiratory rate was in every case from 4 to 10 per minute less, the week following vagotomy.

In the case of the previously severed vago-sympathetic nerve trunk in the ewe examined, it was found that ligature and cutting followed by stimulation of the peripheral end with weak and strong induced current produced no effect upon the heart rate. This would lead to the conclusion either that there is no regeneration of the efferent autonomic fibers of the vagus in the sheep or that regeneration is not complete in 12 months.

It will be noted that in figure 1 the first application of a long-continued stimulus to the peripheral end of this right nerve resulted in a decrease in pulse pressure, indicated by the Hürthle manometer. No change of course was shown by the mercury manometer, since this does not register pulse pressure.

It might be possible to explain the results of Johansson and Tigerstedt (12) in which they found that stimulation of the left vagus of a cat resulted in a decreased force of contraction of the heart, by assuming the presence of more than one kind of fiber in the vagus. If this should be true in the sheep, it is possible that complete regeneration of this type of fiber had occurred and caused the decrease in pulse pressure. This explanation is rendered rather improbable by the work of Erlanger (13).

It is more probable that the escape of this strong stimulus to surrounding tissue caused a momentary increase in vasomotor tonus with a resulting decrease in pulse pressure.

Light stimulation of the peripheral end of the right (normal) severed vagus shows slowing of the heart. It is the typical picture of cardiac inhibition with vagus escape (see fig. 2).

The fact that the pneumograph was applied around the abdomen would render questionable the assignment of explanations for the peculiarities of the respiratory curve at the last stimulation of the left vagus. It would be so easy to obtain distorted records through normal functional movements of the abdominal viscera.

Upon stimulation of the peripheral end of the right (normal) vagus, there is a drop in the respiratory curve. This drop may be due to the sudden motor stimulation of the stomach, resulting in violent contraction with consequent traction upon the diaphragm at the cardia. This would increase the size of the abdomen by visceral compression, giving this type of curve.

Digitized by Google

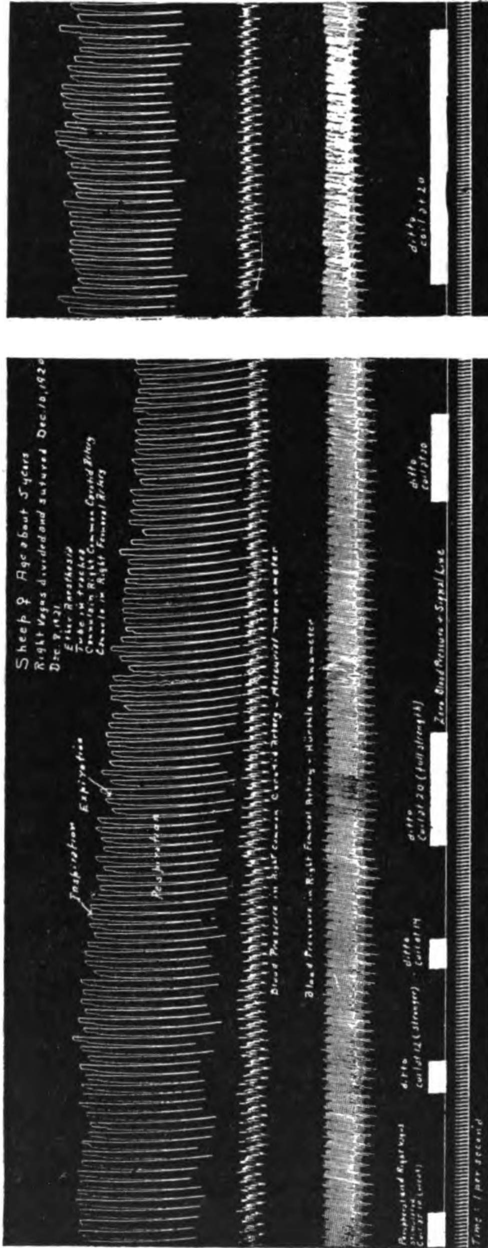


Fig. 1. Blood pressure (Hürthle and mercury manometers) and respiratory tracings during stimulation of the peripheral end of the right (injured) vagus nerve.

Digitized by Google

It will be noted in figure 2 that there was a distinct slowing and deepening of respiration after tying the first ligature on the right (normal) vagus nerve. Although the rate increased slightly within a few minutes, it did not return to normal during the course of the experiment—about 3 hours, nor did the rate increase. This would indicate that in this sheep cutting of the one normal vagus and functional inaction of the other showed the classical respiration picture of double vagot-

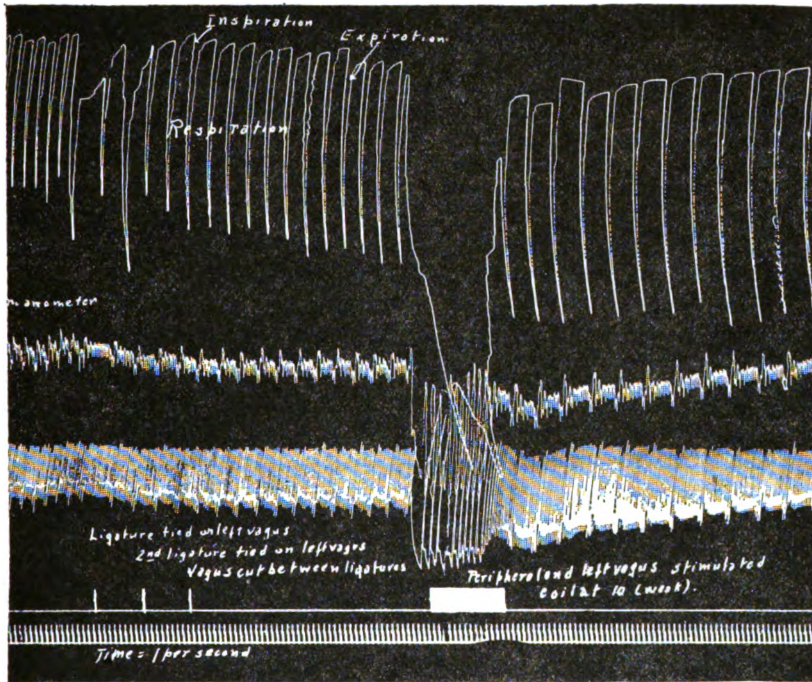


Fig. 2. Blood pressure and respiratory tracings during stimulation of the peripheral end of the left (normal) vagus nerve.

omy, despite the presence of the tracheal cannula. This must be contrasted with the majority of results as reported by Schafer (10) for animals of some other species.

CONCLUSIONS

There is probably no functional regeneration of the autonomic fibers in the right vagus nerve of the sheep within a 12-month period.

Digitized by Google

With a tracheal cannula inserted, the cutting of the second vagus nerve gave the "classical" picture of respiration rate and amplitude following double vagotomy.

I wish to thank Dr. Sutherland Simpson for help in carrying out these experiments, and Mr. B. R. Macmillan, department mechanician, for assistance with the apparatus. I also wish to acknowledge my indebtedness to Mr. S. H. Bassett for assistance at the operations.

BIBLIOGRAPHY

(1) GLUCK: Arch. f. Path. Anat. u. Physiol., 1878, lxxii, 624.
(2) VANLAIR: Arch. d. Physiol. Norm., vi, 1894, series v, 217.
(3) TUCKETT: Journ. Physiol., 1895–96, xviiii, 267.
(4) TUCKETT: Journ. Physiol., 1899–1900, xxv, 303.
(5) STEWART: This Journal, 1907–08, xx, 407.
(6) ROGERS: This Journal, 1920, liii, 15.
(7) LANGLEY: Journ. Physiol., 1895, xviii, 280.
(8) TSUKAGUCHI: Quart. Journ. Exper. Physiol., 1915–16, ix, 281.
(9) SCHAFER AND FEISS: Ibid., 329.
(10) SCHAFER: Quart. Journ. Exper. Physiol., 1919, xii, 231.
(11) COHN: Arch. Int. Med., 1920, xxvi, 105.
(12) JOHANSSON AND TIGERSTEDT: Mitt. a. d. physiol. Laboratorium in Stockholm, 1889, vi, no. 8. Quoted by COHN: Journ. Exper. Med., 1912, xvi, 732.
(13) ERLANGER: This Journal, 1905, xiii, 372.

Digitized by Google

THE SECRETORY ACTION OF THE PANCREAS IN RELATION TO THE THYROID GLAND

I. The Effect of Thyroid Feeding in Rats upon the Secretory Action of the Pancreas

HIROTOSHI HASHIMOTO

From the Laboratory of Prof. K. Miura's Clinic, Medical Department, Tokyo Imperial University, Japan

Received for publication November 19, 1921

It has been noticed by a number of investigators that thyroid-feeding produces certain effects upon the digestive tract of animals. Carlson (1) and Farrant (2) observed in thyroid-fed animals diarrhea and a hemorrhagic condition of the intestines in spite of the increase in appetite. Schafer (9) found that thyroid-feeding causes in growing white rats an increase of food-consumption and an acceleration of bodily growth. Hewitt (4) noticed that the food-consumption of white rats diminished, when comparatively large quantities of thyroid were given, while on the contrary comparatively small quantities caused the animals to consume more food and gain more weight in body than normal. Marbé (7) observed that thyroid administered to dogs acted as a stimulant to the secretory action of the intestinal glands, the quantity of the intestinal juice secreted increasing markedly. Nürenberg (8) fed iodo-thyro-globulin to dogs, and found the secretion of the intestinal and pancreatic juices accelerated and augmented during digestion and for a while after, whereas such changes were not shown by the control animals, which were fed with inorganic iodine compound. A hemorrhagic condition of the intestinal mucous membrane appeared in these thyroid-fed animals at the same time. Kojima (5) found in the pancreas of thyroid-fed white rats morphogenetic changes indicative of rapid multiplication of the gland-cells and at the same time considerable diminution of the zymogen-granules. Determining the amylase-content of the pancreas in thyroid-fed white rats I (3) attempted to investigate the changes in the secretory action of the pancreas resulting from thyroid-feeding. And I observed that thyroid-feeding resulted in a marked decrease of amylase-content of the pancreas.

Digitized by Google

In view of Hewitt's observations of the changes in appetite resulting from feeding different amounts of thyroid, however, it was thought well to investigate further how widely different effects upon the secretory action of the pancreas would be caused by different doses of thyroid administered. Hence in the present investigation white rats of nearly similar size were divided into a number of groups to be fed with different quantities of thyroid, and the changes of the secretory action of the pancreas resulting from thyroid-feeding in the animals of different groups were compared with one another.

Method. The rats employed for experiments were all fully grown ones which fed on mixed bread and milk in equal amounts and greens. They were kept in wire-net cages, being separated from one another. Thyroid administered was desiccated thyroid powder containing 0.488 per cent of iodine, I myself made from fresh ox thyroid glands, and various amounts of it—from 0.5 gram to 0.03 gram per day to each animal—were added to the bread-and-milk mixture. To the control animals were given similar amounts of desiccated beef. The effects of thyroid-feeding upon the general conditions of the animals were observed carefully, viz.: changes of body-weight, general behavior, appetite, excreta, etc. After thyroid was fed for a certain length of time, the animals were killed by chloroform and bled. Immediately after death the body-length was measured, i.e., the distance from the tip of the nose to the center of the anus.

The pancreas was removed in one piece, freed from the adjacent tissues, weighed, and then rubbed in a mortar with fine sand and saline solution. The mixture was rinsed into a flask with more saline solution, using in all a hundred parts by volume (cc.) of saline solution to one part by weight (gram) of gland substance. The flask was plunged into a water-thermostat at 38°C. After an hour the mixture was filtered through paper, resulting in a clear or slightly opaque solution. The extract of pancreas thus obtained was used for determining its amylase-content.

The small intestine in its entire length was removed, ligatures being at both ends, and was slit up with a pair of scissors. Its contents *in toto* were removed, weighed, and mixed in a mortar with the saline solution in the proportion of one part by weight (gram) of juice to ten parts by volume (cc.) of saline solution. The mortar containing the mixture was set in an ice-box for an hour, and then the homogeneous mixture was filtered through paper. The solution of intestinal juice thus obtained was used for determining its amylase-content. The

Digitized by Google

saline solution used was the physiological saline solution, to which was added one-fifteenth part of the solution consisting of one part of a normal solution of acetic acid and thirty-two parts of a normal solution of sodium acetate.

The quantitative determination of amylase was made according to Wohlgemuth's method. The starch-solution used was of 1 per cent. It was made of Merck's soluble starch instead of Kahlbaum's, inasmuch as the former had proved to give results similar to those of the latter. The duration of digestion was limited to 30 minutes, so that the spontaneous deterioration of amylase might be avoided as much as possible. As for formulating the results obtained by this test, the amylolytic activity would be expressed by the amount (cc.) of 1 per cent starch-solution digested by 1 cc. of ferment-solution during 30 minutes at a temperature of 38°C. In order to indicate the quantity of amylase in the following accounts of my experiments, the unit was fixed at such an amount of amylase as would digest 1 cc. of 1 per cent starch solution completely during 30 minutes at a temperature of 38°C.

Results. The amylase-content of the pancreas in sixteen normal, fully grown, male, albino rats quoted as the controls was determined quantitatively to be as following. The amount of amylase per 1 gram of gland substance varied from 21,700 to 53,400 units, and averaged 30,900 units. The amount of pancreas-amylase per 100 mm. of body-length ran from 8,700 to 20,000 units, and that per 100 grams of body-weight, from 7,090 to 15,900 units, the average being 13,300 and 11,400 units respectively. The amylase-content of the pancreas in four control female rats (non-pregnant) varied from 20,800 to 50,000 units per 1 gram of gland-substance, and averaged 31,000 units; the amount of pancreas-amylase per 100 mm. of body-length, from 10,740 to 18,500 units, the average being 13,300 units, and that per 100 grams of body-weight, from 9,790 to 23,750 units, the average being 14,900 units.

As the result of feeding rats comparatively large doses of thyroid—from 0.2 to 0.5 gram of desiccated thyroid daily to each, viz., from 0.08 to 0.21 gram of desiccated thyroid per 100 grams of body-weight—the amylase-content of the pancreas both in the males and in the females was notably diminished. In the majority of these cases the amylase-content of the pancreas was found to have decreased far beyond the lowest limit of the normal variation as shown by the control animals. The percentage reduction in the amount of amylase per unit-weight of gland substance, viz., the concentration or density of amylase in the gland substance, averaged from 55 to 80 per cent in

Digitized by Google

TABLE 1

The average amylase-content and the average weight of the pancreas in the thyroid-fed rats grouped according to dosage of thyroid

LOT, SEX	NUMBER OF RATS IN EACH LOT	THYROID-FEEDING		AVERAGE FINAL BODY LENGTH	AVERAGE FINAL BODY WEIGHT LENGTH RATIO	AVERAGE LOSS OF BODY WEIGHT PER 10 DAYS	PANCREAS		
		Dose per day	Average duration in day				Average weight per 100 mm. body length	Average amylase content: Per 1 gram gland substance	Average amylase content: Per 100 mm. body length
		grams		*mm.*			*grams*		
I M	6	0.5	9	200	0.96	(−19)	0.388 (−11)	13,300 (−57)	5,600 (−58)
II M	2	0.3	11	187	0.82	(−16)	0.486 (+11)	6,300 (−80)	3,100 (−77)
III M	2	0.2	5	198	1.10	(−43)	0.410 (−6)	13,900 (−55)	5,800 (−57)
IV M	2	0.1	15	192	0.82	(−19)	0.488 (+11)	33,300 (+8)	16,100 (+21)
V M	4	0.03	112	198	1.00	(−1)	0.457 (+4)	30,900 (±0)	14,400 (+8)
VII M	16	Controls		206	1.14		0.438	30,900	13,300
VIII M	6	0.3	11	190	0.88	(−20)	0.620 (+35)	12,600 (−59)	8,000 (−39)
IX F	5	0.03	82	188	0.96	(−2)	0.546 (+19)	32,400 (+4)	18,100 (+37)
X F	4	Controls		191	0.95		0.457	31,000	13,200

* The unit of quantity of amylase is fixed at such an amount of amylase as would digest 1 cc. of 1 per cent starch solution completely during 30 minutes at a temperature of 38°C.

The figures in brackets indicate the percentage differences from the control values.

each lot. In the extreme case, the decrease amounted to about 95 per cent of the average amylase-content as shown by the controls, and to 94 per cent of the low limit-value of the controls. The amount of pancreas-amylase per 100 mm. of body-length or per 100 grams of body-weight was similarly reduced, most of the figures obtained for those

Digitized by Google

being far lower than the lowest figure given by their controls. Since thyroid-feeding interfered considerably with the normal gain of body-weight in these cases, the comparison of the amount of amylase per unit of body-length probably gives a more exact figure of the changes which took place than a comparison in terms of body-weight does. The percentage decrease in the average amount of pancreas-amylase per 100 mm. of body-length obtained for each lot varied from 39 to 77 per cent of the control value. In the extreme case the reduction was 95 per cent of the average control value, and 92 per cent of the lowest control value.

On the other hand, the majority of the animals that had received greatly reduced amounts of thyroid—each 0.1 gram of desiccated thyroid daily (0.05 gram per 100 grams of body-weight) or each 0.03 gram of desiccated thyroid on alternate days (from 0.010 to 0.033 gram and from 0.013 to 0.020 gram per 100 grams of body-weight in the males and in the females respectively)—showed a tendency to have an abundance of amylase stored in the pancreas, although none of them showed greater values than the highest limit of the normal fluctuations as shown by the controls. The average amounts of pancreas-amylase per 100 mm. of body-length for these lots were larger than the averages of the corresponding controls by from 8 to 37 per cent. Since the amounts of amylase per unit-weight of gland substance, viz., amylase-concentration on the gland substance, in these cases, were not at all or only slightly increased—on the average from 4 to 8 per cent—these increases of amylase in the pancreas of these animals must be attributed in no small degree to the enlargement of the gland (hypertrophy), which had been caused by thyroid-feeding. The weight of the glands per 100 mm. of body-length in these thyroid-fed rats averaged from 4 to 19 per cent higher than those of their controls.

For the purpose of making the nature of the changes in the secretory action of the pancreas more obvious, I further made use of the results obtained in determining the amylase-content of the intestinal juice. As is generally known, the amylase contained in the intestinal juice, or at least the largest part of it, originates in the pancreas-secretion. In all mammals the pure intestinal juice obtained from isolated loops of the small intestine contains invertase, maltase and occasionally lactase, but no amylase at all. Throughout the entire alimentary canal amylase is provided by no digestive fluid except saliva and the pancreatic juice. And as stated by Langley (6), the salivary amylase is destroyed eventually by the hydrolic acid contained in the gastric

Digitized by Google

juice, 0.003 per cent hydrolic acid—approximately pH = 3—being sufficient to destroy the salivary amylase. Hence it may be claimed that an increase or decrease in the amount of intestinal amylase indicates an increase or decrease in the amount of amylase given by the pancreas, provided H-ion concentration of the gastric juice be higher than pH = 3, strong enough to destroy the salivary amylase before it enters into the duodenum.

TABLE 2

The average amylase-content of the pancreas and that of the intestinal juice in the thyroid-fed rats grouped according to dosage of thyroid

LOT, SEX	NUMBER OF RATS	THYROID FEEDING		AVERAGE FINAL BODY LENGTH	AVERAGE LOSS OF BODY WEIGHT	PANCREAS			INTESTINAL JUICE		STOMACH CONTENT	
		Dose per day	Average duration in day			Average weight in grams per 100 mm. body length	Average amylase content: Per 1 gram gland substance	Average amylase content: Per 100 mm. body length	Average amount in grams per 100 mm. body length.	Average amylase content per 100 mm. body length	Weight	Acidity
		grams		*mm.*							*gms.*	*pH*
I M	2	0.3	11	183	(−18)	0.486 (+8)	6,300 (−80)	3,100 (−76)	0.368 (+26)	118 (−53)	2.5	3.5
II F	2	0.3	17	186	(−19)	0.767 (+70)	16,800 (−45)	12,100 (−8)	0.450 (+55)	580 (+129)	0.5	1.5
III M	2	0.2	5	198	(−17)	0.416 (−7)	13,500 (−56)	5,600 (−68)	0.530 (+82)	338 (+46)	1.6	2.0
IV M	2	0.1	14	192	(−27)	0.489 (+9)	33,300 (+8)	16,000 (+21)	0.748 (+156)	1,090 (+331)	2.3	3.0
V MF	6	Controls		207		0.450	30,800	13,200	0.291	253	4.0	2.5

See the foot-note of table 1.

In the normal albino rats, when the stomach was filled with a fair amount of food and the small intestine with much turbid and thick chyme, i.e., at the period of intestinal digestion, the amount of amylase contained in the chyme present throughout the entire length of the small intestine varied from 144 to 433 units, and averaged 253 units per 100 mm. of body-length.

Among the thyroid-fed rats, observed under conditions similar to those of the controls, two males receiving 0.3 gram of desiccated thyroid daily for 8 and 13 days respectively, showed fair reductions in the amounts of intestinal amylase, one of which was obviously lower

Digitized by Google

than the minimum limit of normal variations as shown by the controls. The amounts of amylase contained in the pancreases of these two animals were found to be below the minimum limit of the normal fluctuations, both per unit-weight of gland and per 100 mm. of body-length. It is evident that the secretory action of the pancreas was severely impaired in the above cases.

On the other hand two male rats receiving 0.1 gram of desiccated thyroid daily for 10 and 17 days respectively showed tremendous increases in the amylase-content of the intestinal juice. The amounts of the intestinal amylase per 100 mm. of body-length were notably larger than the maximum amount shown by the controls, and averaged over 300 per cent higher than the average of the controls. The amylase-content of the pancreas in these animals was found within the limits of the normal variations as shown by the controls, although the average from these was a little higher than the control value. Such changes of the amylase-content of the pancreas by themselves indicate merely that there might be tendency toward an increase in the secretory action of the pancreas, but the increase of the pancreas-amylase is too small to prove that the secretory action of the gland was increased enormously as the result of thyroid-feeding. Judging from the results of determining the amylase-content of the intestinal juice, however, it is evident that there was a decided hyperfunction of the pancreas in the above cases.

Another interesting result was obtained in two male rats receiving 0.2 gram of desiccated thyroid daily for 3 and 6 days respectively, and two females receiving 0.3 gram of desiccated thyroid daily for 15 days and 19 days respectively. In the former two animals there were fair decreases in the amylase-content of the pancreas—below the limit of normal variations—but the amylase-content of the intestinal juice was by no means decreased, but was found to be within the limits of normal variations. The evidence that the intestinal juice contained a normal amount of amylase argues that the pancreas had been giving out into the intestine as much amylase as normal, although in the pancreas itself, when examined, was found very little amylase stored up. The most reasonable interpretation of the above changes would be that the gland was temporarily exhausted in its secretory action, after it had been functioning vigorously at the period of intestinal digestion. Now in the animals of the latter group the amylase-content of the intestinal juice was found to be rather markedly increased, even by a hundred per cent of the control value in spite of a considerable reduction in the

Digitized by Google

amylase-content of the pancreas. These changes suggest that the pancreas was exhausted in its function of producing amylase, after it had been producing and sending out larger amounts of amylase into the intestine than normal. The pancreas of these animals seems capable of meeting the demand on the part of the digestive tract for digestive ferments with an increased secretion, but it is soon exhausted, i.e., it is in a condition of increased secretory activity associated with increased exhaustibility.

From the above evidences we may infer that feeding animals certain small amounts of thyroid induces a profitable increase in secretory action of the pancreas and causes the gland to produce and send out greater amounts of digestive ferments than normal, but, if the dose of thyroid is increased beyond a certain limit, the gland is impaired, so that it cannot meet successfully the demand of the digestive tract for the ferments. And we may further assume that feeding certain intermediate amounts of thyroid changes the secretory action of the pancreas, so that the demand of the digestive tract for increased quantities of ferments may be met by the gland for a while successfully, but the pancreas itself will be easily exhausted in the process.

SUMMARY

Feeding animals comparatively large quantities of thyroid interferes notably with the secretory action of the pancreas, but on the contrary feeding comparatively small quantities causes a fair increase in that function.

BIBLIOGRAPHY

(1) Carlson, Rooks and McKie: This Journal, 1912, xxx, 129.
(2) Farrant: Brit. Med. Journ., 1913, ii, 1363.
(3) Hashimoto: Endocrinology, 1920, iv, 56.
(4) Hewitt: Quart. Journ. Exper. Physiol., 1915, viii, 113, 297.
(5) Kojima: Quart. Journ. Exper. Physiol., 1917, xi, 255.
(6) Langley: Journ. Physiol., iii, 246; iv, 18.
(7) Marbé: Compt. Rend. Soc. Biol., 1911, lxx, 1028.
(8) Nürenberg: Russki Wratsch, 1912.—Cited in Biedl's Innere Sekretion, 1916.
(9) Schafer: Quart. Journ. Exper. Physiol., 1913, v, 203.

Digitized by Google

THE SECRETORY ACTION OF THE PANCREAS IN RELATION TO THE THYROID GLAND

II. The Effect of Thyroidectomy in Rats upon the Secretory Action of the Pancreas

HIROTOSHI HASHIMOTO

From the Laboratory of Prof. K. Miura's Clinic, Medical Department, Tokyo Imperial University, Japan

Received for publication November 19, 1921

In my previous experimental researches into the effect of thyroid-feeding upon the secretory action of the pancreas, I (3) ascertained that feeding comparatively small amounts of thyroid causes an increase in the secretory action of the pancreas of albino rats, whereas comparatively large amounts of thyroid induce rather a decrease in the same. Now the question arises: Does the active principle of thyroid gland in the normal condition of the animal-organism also act as a hormone or an excitatory autacoid (6), increasing the rate of the action of the pancreas so as to maintain its normal secretory function? Do such amounts of thyroid-autacoid as are passed by the normal gland into the general blood-circulation exert an excitatory effect upon the secretory action of the pancreas? In solution of this problem, we need additional evidence to support the above data derived from the experiments in thyroid-feeding, inasmuch as many poisonous agents act as a stimulus to various physiological functions of the animal-organism when administered in very small doses, but are injurious when given in larger amounts. In order to establish the point in question, we must resort first to another method usually employed by investigators for the purpose of determining the function of the endocrine organs, viz., observation of the changes in the secretory action of the pancreas resulting from the surgical removal of the thyroid gland. If the active principle of the thyroid gland promotes the secretory action of the pancreas even in the normal condition of the animal, it may be expected that the absence of the thyroid gland would lead to an impairment of that action. The following experiments were made to ascertain the effect of thyroidectomy upon the secretory action of the pancreas.

Digitized by Google

Albino rats were employed for the experiments. The dietetic and environmental conditions of the animals during the experiments and the methods of determining the secretory action of the pancreas were altogether identical with those of my previous experiments. The thyroid glands were removed without anesthesia after keeping the animals for at least 2 weeks under the same dietetic conditions as those following the operation. When the parathyroids were found lying on the surface of the thyroid glands, these were removed and grafted deep in the operative wounds. The wounds usually healed without suppuration within 2 days. Thyroidectomized animals were killed at various periods after the operation, and the absence of the thyroid glands was verified by naked eye. When any formations simulating thyroid glands were discovered, these were examined microscopically.

The first series of experiments was performed on albino rats of four litters. Some of the operated ones showed symptoms of *tetania parathyreopriva* which usually appeared 2 or 3 days after the operation, stiffness and quivering of the limbs, and, occasionally, rapid, gasping respirations. Such animals died eventually, usually within 5 days. Only one of these lived as long as 18 days, when it was killed. A large number of the animals was lost after the removal of their thyroid glands without exhibiting tetany, so that only seven were available for measuring the secretory action of the pancreas. These animals were killed 15 and 17 days respectively after the operation. The thyroidectomized adult animals appeared to be entirely normal in the secretory action of the pancreas as well as in their general condition, no difference from their controls being evident. Among young adult animals the thyroidectomized one lost not a little of its body-weight, but its pancreas was apparently normal in its secretory action. In one rat which had exhibited the symptoms of chronic tetany, the pancreas and the intestinal juice contained fair amounts of amylase.

Judging from the results of the above experiments made on animals killed as late as 15 or 17 days after the removal of their thyroids, we are forced to conclude that the lack of thyroid secretion does not induce any changes in the secretory function of the pancreas. Cramer and M'Call (2) in their investigations of the effect of thyroidectomy in albino rats on the gaseous metabolism, ascertained that the metabolism after removal of the thyroid glands passes through two stages: At first, there is a reduction of the metabolism which is still reduced in the second week after the operation; and, later, an increased metabolism, produced probably by way of compensation. They regarded it as

Digitized by Google

reasonable to restrict the term "experimental hypo-thyroidism" to the early period in which the metabolism is reduced, as is usually seen in the pathological hypo-thyroidism of human subjects. If such a compensatory arrangement can be brought about so soon after the removal of the thyroid glands in albino rats, as demonstrated by the above investigators, then the results of my first series of experiments, as described above, are of little value in determining the changes in the secretory action of the pancreas due to the absence of thyroid secretion because, in order to measure the secretory action of the pancreas, the animals in the above experiments were killed more than 15 days after the removal of their thyroid glands, by which time the compensatory adjustment for the loss of the thyroid glands would already have been made.

Hence in the second series of experiments the animals were killed at earlier periods as well as at later periods after thyroidectomy, one of these being killed as early as 2 days after the operation, when the wounds had just healed. The animals employed were all fully grown male albino rats, taken from closely related litters of the same strain. After the operation comparatively few animals—about 20 per cent of the operated ones—suffered so severely from the operation itself as to be incapable of taking food properly on a subsequent day. The majority—55 per cent—lost their appetite rather later on the third day after the operation, when the wounds had completely healed. Nearly at the same period the animals became slightly inactive in general behavior. In none of these thyroidectomized animals, killed within 13 days after the operation, were thyroid glands found to be regenerated. But the animals, if killed more than 15 days after the thyroidectomy, exhibited in the neighborhood of the larynx small round or oval bodies that were about ¼ to ½ of the normal thyroid gland in size and consisted of tissue quite similar to that of the normal thyroid glands. It was not certain whether the appearance of these formations was due to the regeneration of the gland tissue from the remnants of the removed glands, or hyperplasia of the accessory glands.

As to the secretory action of the pancreas, only the results obtained in the second series of the experiments were shown in the table. In an animal killed 2 days after the operation, when the wound had just healed, the amylase-content of the pancreas as well as of the intestinal juice was found to be quite large. In two animals killed 3 days after thyroidectomy the secretory action of the pancreas was found to be considerably reduced, one especially of these showing the amylase-

Digitized by Google

content of both the pancreas and the intestinal juice to be clearly less than the least shown by the controls. An animal killed at the end of the first week exhibited similarly a fair reduction. Comparing the

TABLE 1

Average amylase-content of the pancreas and that of the intestinal juice in thyroidectomized rats and their controls

	Week, in which rats were killed, after operation	Number of rats	Average final body length	Average final body weight length ratio	Pancreas: Average weight per 100 mm. body length	Pancreas: Average amylase content: Per gram gland substance	Pancreas: Average amylase content: Per 100 mm. body length	Intestinal juice: Average amount in grams per 100 mm. body length	Intestinal juice: Average amylase content per 100 mm. body length	Stomach content: Weight	Stomach content: Acidity
			mm.		grams					grams	pH
Thyroidectomized ...	First Second	4	185	0.785	0.275	33,400 (−43)	9,400 (−49)	0.713	545 (−37)	0.5	2.5
	Second	4	182	0.710	0.339	52,300 (−9)	17,600 (−2)	1.000	660 (−31)	1.4	2.8
	Third	3	183	0.818	0.343	58,100 (+17)	20,000 (+13)	1.078	1,050 (+11)	3.2	2.8
Controls	First	2	189	0.810	0.319	58,600	18,400	1.165	865	1.0	1.0
	Second	4	180	0.728	0.307	57,800	18,000	0.785	964	0.6	2.8
	Third	3	191	0.860	0.355	49,700	17,700	1.140	945	1.7	2.8
Thyroidectomized...	First Second	8	183	0.748		42,000 (−26)	13,500 (−26)		603 (−35)		
Controls	First Second	6	183	0.756		58,100	18,150		932		

The unit of quantity of amylase is fixed at such an amount of amylase as would digest 1 cc. of 1 per cent starch solution completely during 30 minutes at the temperature of 38°C.

The figures in brackets indicate the percentage differences from the control values.

average amylase-content of the pancreas and the intestinal juice in all the animals killed within a week after thyroidectomy, with that in their proper controls, the reduction was estimated as of 49 and 37 per cent respectively.

Digitized by Google

In four animals killed in the second week after thyroidectomy, the amylase-content of the pancreas was found to be within the limits of normal fluctuations as shown by their controls, and the average figure differed but little from that of their controls. We may assume, however, that there was a fair reduction in the secretory action of the pancreas, for it was found that the amylase-content of the intestinal juice tended to decrease in the thyroidectomized animals, and the average was less by 31 per cent than that of the controls.

In three animals killed the third week after thyroidectmy, the amylase-content of the pancreas and the intestinal juice was found to be somewhat larger than that of their proper controls. As these animals all, however, exhibited regenerated thyroid glands in the place of the removed ones, it need hardly be pointed out that the observation of the secretory action of the pancreas in such animals can give us little information concerning the changes due to the absence of the thyroid-secretion.

The changes resulting from the absence of the thyroid-secretion should be expected only at an early period within 2 weeks after the removal of the thyroid glands, when no compensatory adjustment has yet been made. The average amylase-content of the pancreas, per unit-weight of the gland as well as per 100 mm. of body-length, in all the thyroidectomized animals, killed within 2 weeks after the operation, was less than that in their controls by about 26 per cent. The average amount of intestinal amylase per 100 mm. of body-length in the same thyroidectomized animals was less by 35 per cent than that in their controls.

Summary. The removal of the thyroid glands in albino rats causes a decrease in the secretory action of the pancreas, the change being evident within 2 weeks after the operation, when no compensatory mechanism for the lost glands has yet been evolved.

CONCLUSION

Summing up all the evidences that I have been able to obtain in the course of my investigations into the effect of thyroid-feeding as well as thyroidectomy upon the secretory action of the pancreas, I may state the following conclusions: The secretory action of the pancreas varies considerably according to the amounts of thyroid-secretion circulating in the body. A moderate augmentation of the thyroid-secretion in the body provokes the hyper-functioning of the pancreas, whereas a lack of the thyroid secretion induces a reduced activity of the gland. As to the part played by the thyroid-secretion in the normal functioning

Digitized by Google

of the pancreas, we may infer that the thyroid-secretion acts as an excitatory autacoid upon the pancreas in maintaining its normal activity.

Further investigations must decide the question how the excitatory effect of the thyroid-autacoid upon the secretory action of the pancreas differs from that of *secretin* which has generally been regarded as an excitatory hormone acting as a specific stimulus to the pancreas-cells—Bayliss and Starling (1). It has long been a generally accepted theory that the thyroid-secretion acts as a stimulus to oxidation. Furthermore, according to Herring (4), the thyroid-secretion stimulates the suprarenal glands and causes them to produce increased amounts of adrenalin. Koopman (5), moreover, found that the thyroid-autacoid can cause an increased formation of antibody in the serum. A similar excitatory effect we see now in its action upon the secretory function of the pancreas. In view of these evidences, it may be concluded that the thyroid hormone does not act as a specific stimulus to a particular organ or a particular physiological function, as does the *secretin* to the secretory function of the pancreas, but that it probably acts as a non-specific stimulus generally upon various physiologic functions of the animal-organism.

The experimental work was done in the laboratory of Professor Miura's Clinic. I am much indebted to him for his care and supervision. My hearty thanks are due to Prof. M. Kojima (in the Naval Medical Academy) for his help.

BIBLIOGRAPHY

(1) Bayliss and Starling: Journ. Physiol., 1902, xxviii, 325.
(2) Cramer and M'Call: Quart. Journ. Exper. Physiol., 1918, xii, 81.
(3) Hashimoto: This Journal, 1922, lx, 351.
(4) Herring: Quart. Journ. Exper. Physiol., 1916, ix, 391.
(5) Koopman: Endocrinology, 1919, iii, 318.
(6) Schafer: Endocrine organs, London, 1916, 6.

Digitized by Google

A STUDY OF WEIGHT REGULATION IN THE ADULT HUMAN BODY DURING OVER-NUTRITION

ADDISON GULICK

From the Laboratory of Physiological Chemistry, Department of Physiology University of Missouri, Columbia

Received for publication December 3, 1921

In contrast to the brilliant successes that science has won in the study of nitrogen equilibrium, there is a rather discouraging inconclusiveness in the work that has been done on the factors that regulate the nutritive balance of the fat tissue of the body, and the balance between the total intake and total output of potential and kinetic energy in the adult warm-blooded organism. We do not yet know what mechanism there is to prevent the unlimited accumulation of potential energy in the form of an overload of adipose tissue. Is nerve regulation through changing appetite the only guide, or does the body vary its destruction of fats and carbohydrates in accordance with their fluctuating intake, somewhat after the manner that it varies its nitrogen exchange?

The best road to a true solution is probably in a study of selected contrasting individuals of the over-fat and under-fat body habits, or better still, of the easily fattening and difficultly fattening types.

The problem was first drawn to my attention by observation of myself, and the experiments here reported were performed upon myself as a selected example of the "spare" or apparently non-fattening type. I had long noted my inclination toward a very copious diet of predominantly starchy nature, in spite of which my weight remained fairly constant, even on a moderate round of activity, at a figure well below the average for my stature. If the hereditary constitution is important in this connection, such family data as I possess seem to indicate that I am derived largely from non-fattening strains.

It has long been recognized that food intake has a powerful effect on the rate of oxidation in the body. In the case of protein food, Rubner (1) distinguishes two such effects. The first of these is the oft discussed specific dynamic effect. The second is a change which appears

Digitized by Google

more gradually, and is explained by him as a stimulus from plethora of nitrogenous products in the cell fluids. The specific dynamic effect occurs equally in well-fed and badly-fed animals, and so it cannot function as a safety valve for excessive intake. But the secondary or plethora effect may very well so function. According to Rubner, this effect shows itself as a cumulative increase in the specific dynamic effect of heavy protein meals, when they are administered on a series of days. He states that in spite of its cumulative character, this secondary effect is not in evidence during the hours when no food is being absorbed. Consequently a dog that has been over-fed in this manner shows no change in its basal metabolic rate, as ordinarily determined on an empty stomach (p. 260, loc. cit.).

Similar results are reported by Dengler and Meyer (2) in their study of the basal metabolism of a man who was over-fed with protein. They found the basal rate changed to a surprisingly slight degree as a result of nitrogen accumulation, and hence conclude that the excess of stored nitrogen was in a non-stimulating form.

A. Müller (3), in tests on a young man, found that the secondary rise on a high protein diet develops rapidly, before much nitrogen storage can have occurred, and then fails to keep pace with the stored nitrogen, and is at best trivial in proportion to the quantity of nitrogen that is finally accumulated. So he does not look upon this heightened catabolism as a result of the stored nitrogen.

Grafe and Koch (4) experimented upon persons who entered a clinic in an extremely under-nourished condition. The two principal subjects were both put through a heavy feeding period of 7 weeks' duration to bring them back to normal weight. There resulted a very notable increase in the gaseous exchange. Thus the adult subject, at the initial weight of 40.3 kgm., showed a basal metabolism of 1081 cal. (26.8 cal. per kgm.) and a dynamic effect from eating a meal equal to 24 per cent of the calorie value of the meal. Seven weeks later at a weight of 60 kgm., the basal metabolism had risen to 1946 cal., (32.3 cal. per kgm.) and the dynamic effect had risen to 32 per cent of the fuel value of the meal. Most of the gain in basal metabolism occurred near the middle of the experiment. Although it appeared less rapidly than in Müller's experiment, it was of greater magnitude. The tests of the dynamic effect of the mixed diet fluctuated widely, and cannot be easily interpreted.

Atkinson and Lusk (5) experimented along lines similar to those followed by Rubner, but by over-feeding to a greater extreme were

Digitized by Google

able to report that prolonged and excessive meat diet can cause a rise of the dog's basal metabolism which lasts for as much as two and one-half weeks after the special diet is ended. For their case, then, they conclude that the excess nitrogen was accumulated in a stimulating form.

The converse to the idea of increased or spendthrift oxidation during over-nourishment, is the idea of an especially economical oxidation during under-nutrition.

Anderson and Lusk (6) studied the respiration and the treadmill efficiency of a dog working with empty stomach, with definite meals, and during a prolonged fast. Among other things it was found that the markedly lowered basal metabolism of the fasting period, with its economy of nourishment, carried over into the period immediately following the fast. Eighteen hours after the first liberal meal the metabolism was at essentially the same base level as during the fast, showing that the total condition of the body, and not the question of a large or small influx of food on the previous day, determined the height of the basal metabolism. It is thus to be noted that this dog showed a persistent economy of metabolism after a period of fasting, comparable to the period of wasteful metabolism which Atkinson and and Lusk's dog showed subsequent to a course of over-feeding.

The period of the World War has brought out a series of papers on gaseous metabolism during prolonged under-nutrition. Zuntz and Loewy (7), (8), who have a series of records of their basal metabolism since 1888, studied the effect on themselves of eating the German war ration, practically without any of the additions that free use of money could furnish. On this diet, which was inadequate in fuel value, and especially deficient in protein, they report in the case of Zuntz a 10 per cent fall of basal metabolism below the previous average, and in the case of Loewy a fall of 16 per cent. This decrease is reported in terms of calories per day per square meter by the Meeh formula. The more accurate DuBois formula would show even greater percentage changes in rate. Muscular efficiency on the treadmill had gone down in comparison to previous tests.

F. G. Benedict, Miles, Roth and Smith (9) worked on the metabolism of volunteer squads of young men during experimental under-nutrition which covered three months of low diet. They found a very remarkable fall in the total fuel needs. Starting at an unknown high level which was above 3000 cal., and may have been as high as 3800 cal., they were finally able to hold their weight constant on an average net intake per

Digitized by Google

person of 1950 cal., the fecal and urinary calories being estimated and deducted. Meanwhile there had been a notable loss of nitrogen, 130 grams previous to the first serious interruption (at Christmas) and further losses to the end of the experiment. The basal metabolism fell from an average of 1686 cal. at the start to 1367 cal. at the lowest point, or from 940 per sq. m. to 788 per sq. m. Thus the absolute figures fell 20 per cent and the rate per square meter came down 16 per cent. A remarkable slowing of the pulse accompanied this change. Efficiency in the tests of mechanical work was not impaired. The authors ascribe the changes to the withdrawal of the influence of dispensable nitrogen from the body, and argue that no great inroads had been made on the essential protoplasm.

Joffe, Poulton and Ryffel (10) report upon a case of extreme undernutrition in a vegetarian, who had probably previously habituated himself to a very meager intake. Throughout the tests the basal rate stayed in the neighborhood of 26.6 cal. per square meter per hour, or 638 per square meter per day, calculated according to DuBois. The increment of oxidation during work was about average, showing that the man had about the average of calorie efficiency in work. His pulse was always below 50 in the reclining position.

Investigators agree, then, that when the diet is varied downward there is a factor or group of factors tending to adjust the calorie output to the intake.

All the researches thus far reviewed follow the method of gas analysis, and judge the balance between intake and output so far as possible by a direct measurement of both. Another plan of procedure is to depend upon the body weight as the criterion to show whether a balance of intake and output has been established. In order to obtain convincing results by this plan it is necessary to let the tests cover long periods of time, and also to have very large differences in the measured diets of the different experimental periods, so as to far outweigh any variations in energy expenditure that may come from uncontrolled factors. In the past this general mode of experiment has been used, either with or without a supplementary study of the gas metabolism, chiefly by Neumann and by Grafe and his pupils.

Neumann (11) carried out upon himself one of the most prolonged quantitative diet experiments ever recorded, and showed a food intake which gave averages on different years of 2427 cal. and 2057 cal. respectively per 70 kgm. body weight. The actual weight in the former test (1895–96) averaged 66.5 kgm., and in the latter test (1900–01)

Digitized by Google

averaged 72 kgm. In both years the weights were virtually stationary, tending slightly upward. Neumann's results seem to show ability of the organism to stabilize its weight on either low or medium intakes of fuel. But they do not deal in extreme differences of diet.

Grafe and Graham (12) carried out a prolonged experiment upon a dog, keeping full account of the nitrogen metabolism and weight during very marked over-feeding. From time to time the gas exchange was also determined. Although prevented from taking much exercise, this dog showed extraordinary constancy of body weight during both normal diet and excessive feeding. A puzzling feature is the fairly moderate gas exchange which the dog showed in all the tests.

It might be hoped that comparison of the metabolism in abnormally obese individuals and in persons of normal body habit might throw some light on the factors that prevent most persons from fattening indefinitely. This aspect of the problem was studied by Rubner (13), by A. Magnus-Levy (14) and by von Noorden (15). As summarized by von Noorden, the weight of evidence in these earlier papers is for a fairly comparable metabolic rate in these and the normal cases. DuBois and his colleagues improved this observation by applying new methods for determining the surface area of the human body. (See James H. Means (16), and F. C. Gebhart and Eugene F. DuBois (17).) Using DuBois' determinations of surface, it is easily shown that the great majority of over-fat subjects have a basal rate falling well within the normal rate per square meter of surface. This establishes the fact that the laying on of fat is not caused by a depression in the basal rate. The alternatives still left to account for an obese human type are *a*, an unproved possibility, referred to by von Noorden, that without having a lowered basal rate, the obese may still show an exceptionally low cost of digestion, perhaps by not showing the full normal specific dynamic effect of foods; or *b*, that they partially or entirely lack Rubner's "secondary effect," which causes an upward shoving of the specific dynamic effect, and sometimes even of basal metabolism, whenever the protein over-nutrition becomes cumulative; or *c*, that control over fattening is not referable to any alteration of basal metabolism nor of the energy cost of digestion, but is to be sought in some such factor as a changed appetite.

We may sum up from the literature that under-nutrition (with loss of nitrogen) has a marked lowering effect on the basal metabolic rate and on the total metabolism of the twenty-four hours. Over-nutrition, coupled with heavy enrichment of the body with nitrogen, has at

Digitized by Google

least in some instances an effect on the basal rate, and has been repeatedly found by Rubner and others to push up the specific dynamic or stimulating effect of protein during absorption, to higher and higher figures. Whenever these factors are at work they all tend to limit the fluctuation of the body mass, and especially to limit the accumulation of body protein. Grafe and his collaborators are the only ones who have argued that a similar type of factors is powerful in preventing the immoderate accumulation of body fat.

Outline of experiments. The general intention of the experiments here reported was to attempt to establish constancy of weight at various levels of total intake. In order to insure the adequacy of the protein and accessories throughout the experiment, milk and eggs figure in all the dietaries used. Small amounts of fats were used but excesses were avoided because it is too easily conceivable that fats might be shunted into the adipose tissue, making a passive increase of body weight without in any way having shared in the metabolism. The main source of nourishment was carbohydrate, from rice, wheat and oats, and the experiments consisted chiefly in varying the quantity of starchy food from these sources.

The first test in March, April and May, 1916, was to find the minimum diet that would maintain an approximately normal body weight. After that the intention was to establish a constant weight on a high level of exchange. This attempt lasted with interruptions from May, 1916, to July, 1917.

At the height of this period of maximum weight and food intake the basal metabolism was determined by the analysis of gas exchange, conducted at the Carnegie Nutrition Laboratory, through the courtesy of Dr. F. G. Benedict and Dr. Thorne M. Carpenter. The body weight was then brought back rather abruptly to the initial level by means of a low calorie diet in July and August, 1917. To conclude the experiment another determination was made of the quantity of food necessary to hold the weight constant.

During several of the above periods records were made of general physical activity. Data were taken for the nitrogen balance during the period of rapid reduction. Analyses of foods were made for this period. but not for any of the other periods, nor were any tests made of combustion value. Instead the diet was limited to a very small selection of foods that would be as free as possible from erratic fluctuations in composition. This method is undoubtedly liable to a certain degree of inaccuracy, but not, we believe, to major errors

Digitized by Google

TABLE 1

Condensed outline of dietary

	EXPERIMENTAL PERIOD AND DATE								
	I	II		III and IV		V		VI	
	1916 March 4 to 29	1916 March 30 to April 22	1916 April 23 to May 9	1916 May 10 to June 13	1916 June 14 to October 23	1916 October 24 to December 12	1916 December 13 to March 23	1917 March 24 to June 4	1917 June 5 to July 7
Rolled oats, gram (air dry	0	0	0	0	Ate heartily of varied and liberal diet; outdoor life for 2¼ months.	80	On training; under-slept, under-ate, and lost weight at first, then regained the weight gradually on a liberal diet.	100	Diet irregular but essentially like Period VI. (Respiratory analyses).
Rice, gram (air dry))	300	300	300	325		200		200	
Milk, cc	1450	1450	1450	1450		1450		1450	
Butter, gram	30	30	35	50		50		50	
Rolls (40–50 grams each)	Two	Two	Two	Six to ten		Six to eight		Six to eight	
Eggs (45–55 grams each)	Two	Two	Two	Two		Two		Two	
Sugar, gram	0	0	0	30 grams some days		0		0	

	EXPERIMENTAL PERIOD AND DATE									
	VII	VIII		IX					X	XI to XIII
	1917 July 8–14	1917 July 15–25	1917 July 26 to September 1	1917 September 2–5	1917 September 6–8	1917 September 9	1917 September 10 to October 3	1917 October 9–12	1917 October 13–23	1917 October 24 to November 27
Rolled oats, grams (air dry)	100	65.9	30	47.5	100	100	100	100	100	100
Rice, grams (air dry)	200	96.4	60	95	200	200	200	200	200	200
Milk, cc	1402.7	1541.9	1417.5	1402	1393	1393	1393	1431	1431	1431
Butter, grams	40 (pure fat)	0	0	0	0	0	0	0	0	50
Eggs (45–55 grams each)	100.2 gm.	251.1 gm.	251.3 gm.	126.5 gm.	Two	Two	Two	Two	Two	Two
Shredded wheat biscuit (about 30 grams each)	375 gm.	6.8 gm. (av.)	0	0	0	One	Two	Three	Four	Four

or large systematic discrepancies that would alter the tenor of the conclusions.

The figures for the calorie values of the foods are taken consistently from Atwater and Woods' tables published by the U. S. Dept. of Agriculture (18). These figures are based on Rubner's standard values for the physiologically available energy in protein, fat and carbohydrates when presented in the digestive tract in "perfectly digestible" form. Up to July, 1917, the foods were all of this "perfectly digestible" form, and it would be correct theoretically to use these fuel values without deducting for the loss by feces. After that date the use of shredded wheat in the diet caused a considerable increase in the moist weight of feces, but apparently not so great a change in the dry weight. The dry weight of a series of feces samples which were closely comparable to those of March and April, 1916 (ration of 2744 cal.), averaged 27 grams per day. This figure comes from an unreported preliminary test made in April, 1915. In June, 1916, it was about 51 grams per day (on 3800 calories), and in July, 1917, it was 78 grams (on 4113 calories with much shredded wheat). In August, 1917, it was about 22 grams (low diet), and in November about 35 grams (3200 calories with medium supply of shredded wheat).

The principal data obtained are the records of weight variation. Every effort was made to obtain reliable and comparable weighings. The regular hour was between 11:30 a.m. and 12:00 m. The fewest possible changes were made in the dietary of the breakfasts, in order to minimize the fluctuations that come from variation in the contents of the digestive tract. Defecation ordinarily occurred in the forenoon, and no weighings are included from the exceptional days on which it had not occurred before the hour for taking the weight. No laxative was used at any time. Care was taken that the bladder should be empty and that the stomach should not contain drinking water at the weighing hour. Whenever the weather was sultry, some water was drunk at about 9:30 to insure against shortage at 11:30. In spite of these precautions there were some rather disconcerting fluctuations of weight, that must be ascribed to variations of water metabolism. Some of the low weights that show abnormally low water content occurred in connection with temporary constipation, and were thus automatically excluded. In cases of insomnia (of which there were several instances toward the end of the experiments) there always resulted an abrupt transitory fall of weight, which is probably chiefly due to an accelerated renal activity. Some of these weights were

Digitized by Google

TABLE 2

Relation between food intake and body weight

	PERIOD AND DATE												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
	1916 March 4–29	1916 March 30 to May 9	1916 May 10–25	1916 May 26 to June 12	1916 October 24 to December 12	1917 March 24 to June 4	1917 July 8–14	1917 July 15 to September 1	1917 September 2 to October 12	1917 October 13–23	1917 October 24–27	1917 October 28 to November 10	1917 November 11–25
Duration in days	26	41	16	18	50	73	7	49	41	11	4	14	15
Initial weight	63.89	62.11	61.60	63.85	70.05	71.20	74.08	74.72	65.14	62.31	61.75	62.00	61.43
Average weight	62.90	61.80	62.76	64.89	70.78	71.81	74.28	68.90	63.75	61.94	61.88	61.49	61.32
Average calorie intake	2733	2744	3480	3806	3376	3545	4113	1874	2441	2781	3183	2970	3204
Deviation of weight from period II	+2%		+2%	+5%	+13%	+16%	+20%	+11%	+3%	+0.2%	+0.1%	−0.6%	−0.8%
Deviation of body surface from period II	+0.7%		+0.6%	+2.1%	+5%	+7%	+8%	+5%	+1.3%	+0.1%	+.04%	−0.3%	−0.3%
Deviation of calorie intake from period II	−0.4%		+27%	+39%	+23%	+29%	+50%	−32%	−11%	+1.3%	+16%	+8%	+17%
Average daily change in weight	−0.68	−0.012	+0.141	+0.129	+0.030	+0.024	+0.091	−0.196	−0.069	−0.050	+0.063	−0.044	−0.003

Digitized by Google

recorded, but care was taken not to make unsuitable use of those particular figures. An abnormally low weight (500 grams below the previous day) was found on November 27, 1917, at the onset of a heavy nasal catarrh. It was discarded, leaving November 26 the last valid weighing. No weights were discarded for any other causes.

It must not be forgotten that body weight is only an approximate criterion for the equality of intake and output. A person who is oxidizing as much as 100 calories (11 grams) of body fat a day, may replace enough of that fat with water and have enough other fluctuations of his water metabolism to completely mask the fact for many days. This makes it very necessary for experiments upon the body weight to cover long periods of time.

Low level tests of 1916. The initial low level tests are not to be understood as representing subnormal nourishment or any great degree of emaciation. At that time (age thirty-four) my weight had been fairly constant for a number of years, between 64 and 66 or possibly 66.5 kgm. This is below the average for the height of 181.2 cm., but if allowance is made for a rather short and narrowly built trunk, it does not necessarily indicate under-nutrition.

No record of activity was made during this period, but the round of occupations corresponded very closely to the recorded activities of May and June, 1917, the schedule of duties, the plan of life and the daily habits being almost identical. This means about five miles a day by pedometer, about forty-five meters of staircases climbed per day, and a variety of light occupations connected with teaching and laboratory, many of which are already included in the estimated mileage. Night hours and habits of sleep were good, averaging not far from 8.2 or 8.3 hours rest per night. It should perhaps be mentioned that I am not a quiet sleeper. By fastening a pedometer to one ankle, it was found that in an average night 150 to 160 movements would be made, of sufficient vigor to be counted as steps by the pedometer. No sports, muscular games or pleasure walks were indulged in during any of the experimental periods upon measured diets. On this quiet manner of life, starting at a weight (on March 3) of 64.16 kgm., it was attempted to establish a nutritive balance at about 2725 calories. This diet was probably a little less than was eaten before the experiments. As long as it lasted the sensations of hunger before meals were more than customarily acute.

For the entire duration of the test, till May 9, the tendency to lose weight was not entirely overcome. The 41-day period, March

Digitized by Google

30 to May 9, 1916 (period II), shows an average daily loss of 12 grams on 2744 calories of food, at an average weight of 61.81 kgm. Thus the body requires more than 2744 calories to sustain it at 62 kgm. even under the very moderate conditions of activity. Assuming that the 12 grams of flesh lost had a calorie value not higher than fat, the daily expenditure was more than 2744 and less than 2855 cal. A similar calculation from the last seventeen days of this period gives an expenditure of more than 2753 and less than 2827 cal.

These figures are distinctly larger than the ordinary expectation at the indicated degree of activity. Based on a height of 181.2 cm. and a weight of 61.8 kgm., the expected output of energy can be listed approximately as follows:

	calories
Basal metabolism, 24 hours[1]	1593.0
Added for 5.8 hours sitting, $\frac{5.8}{24} \times 0.1 \times 1595$	38.5
Added for 10 hours standing, $\frac{10}{24} \times 0.2 \times 1595$	132.1
Added for walking 5 miles, $5 \times 0.6 \times 65.4$ (i.e., clothed weight.)	197.2
Added for estimated activities that fail to show on the pedometer (50 per cent of the walking)	98.6
Added for climbing 45 m. of stairs,[2] $45 \times 4 \times 65.4 \times 0.002343$	27.6
Added for descending 45 m. of stairs,[3] $45 \times 2 \times 65.4 \times 0.002343$	13.8
	2100.8
"Digestion cost" of 2744 cal. food (6 per cent)[4]	164.6
Predictable calorie output	2267.4
Food eaten	2744.0
Discrepancy of intake over predictable need	476.6 or 21 per cent

Where some of these items are problematical, they have been estimated at a rather liberal rate, e.g., the ten hours standing and the unknown activities equivalent to 2.5 miles of walking. The "digestion cost," and the increments of metabolism due to the sitting and standing positions have been placed at figures that may appear rather low, because the data upon these factors published in recent years by F. G. Benedict and his collaborators seem to call for a moderate estimate

[1] Harris and Benedict's prediction for height, 181.2 cm.; weight, 71.81 kgm

[2] Assuming a mechanical efficiency of 25 per cent.

[3] Assuming the descent to cost half as much as the ascent.

[4] Benedict and Carpenter (22).

Digitized by Google

of their effect. (See Benedict and Murschhauser (20); Benedict and Carpenter (22).)

The failure to overcome the negative balance on an intake of 2744 calories indicates a physiological tendency, even at low weight levels, to expend more energy than is to be expected from the list of external activities. This physiological wastefulness left the system slightly under-nourished on a diet which ought otherwise to have been more than adequate.

High level tests. May, 1916, to June, 1917. Periods III and IV of the experiments, covering May 10 to June 13, inclusive, differ from the preceding in an increased amount of carbohydrate with a slight increase of butter (50 instead of 35 grams). In order to avoid overwhelming the stomach with an excessive volume of food, the additions were chiefly to the bread ration rather than the moist cereals. For two weeks (period III) a diet of 3480 cal. was maintained, on which a gain was made of 2.25 kgm. As there was no promise of reaching a balance quickly, a further increase of bread was made and the high feeding was kept up till June 13 (period IV, May 26 to June 13, inclusive). With an average diet of 3806 calories, the daily gains continued about the same, to the final weight of 66.17 kgm.

Periods III and IV can count only as preliminary, being too short to overcome the doubts that are necessarily caused by the fluctuation of water metabolism. It is well known (Bischoff and Voit (23); Voit (24) discussion on page 347) that heavy feeding with a very starchy diet will lead to a notable accumulation of water in the tissues. But we have no basis on which to predict the extent or duration of this process, and so have hardly any clue to the calorie value of the accumulated weight in our experiment. If the accumulation during period IV had been exclusively pure fat,—an assumption which is certainly contrary to fact,— it would have represented a fuel accumulation of 1200 calories per day, or more than the total daily excess of food in calories. The high diet needs to be continued long enough to more nearly stabilize this factor of water intake.

The summer, from June 14 to October 23, was on a liberal and hearty diet. Butter, meat, milk and especially all forms of carbohydrates were supplied unstintingly, and were intentionally taken to the full limit that could be relished. For 2½ months the appetite was under the stimulus of an active outdoor life. The resulting weight of 70.05 kgm. on October 24 was considerably the largest that I had ever reached.

Digitized by Google

There then followed a measured period of fifty days (period V) averaging 3376 calories per day, and with daily activity at most only slightly greater than in the other experimental periods. The average gain during the fifty days was 30 grams per day. This must be interpreted as showing a genuine plus balance, because the gain is distributed throughout the period, including its latter portion when water equilibrium must have been reasonably well established. The small size of the daily gains makes it seem probable that the fuel cost of maintenance and activities had risen along with the body weight.

The period of over-nutrition was unavoidably interrupted in the winter of 1916–1917 by a season of heavy duties, impaired sleep and consequent intolerance for an excessive diet. But there was no interruption of the record of essentially good health, and in February and March good nutritive conditions made it possible to recover the greater part of what had been lost.

On March 24, 1917, period VI was started with the initial weight of 71.20 kgm. It was continued 73 days with an average intake of 3545 calories of food and showed an average gain of 24 grams per day. As in the case of the preceding period, the weight curve does not suggest any modification of the water metabolism by the carbohydrate food. Its greater length renders it a comparatively safe period on which to base calculations. With an average weight only 1.03 kgm. above that of period V the additional 170 calories are carried with a smaller daily gain. There seems, then, to have been a definite although not very great growth of that extravagance in use of fuel food which was noted during period II.

This high expenditure of fuel food is much more pronounced in this period than in any previous test. This is not explained by activity, as the daily habits of period II and period VI are the nearest imaginable approach to duplicates of each other. It is likely that period V had slightly, but only slightly greater physical activity, on account of a slightly heavier schedule of university duties. In that case the relative metabolism of period VI above period V becomes the more noteworthy. Period V is the only one of the first six periods that differed perceptibly in its round of activities.

The record of activities with the aid of pedometers began to be taken that spring, and included about four weeks of period VI. Three pedometers were worn at the start, one at the belt, a second at the right ankle, intended to show minor movements of locomotion, and the third at the right forearm. The forearm position proved to be use-

Digitized by Google

less, because of the changes of posture, and because sudden motions were liable to jam the bob of the pedometer and make it stop recording. The ankle pedometer was also given up eventually. It seemed to be able to record more motions than the belt pedometer during the sitting and standing occupations,but it suffered from very nearly the same mechanical difficulties as the one on the arm. It was read night and morning for 14 days, and supplied the data on restless sleeping to which I have already referred. The belt pedometer was worn only during the waking hours, and was read once daily regularly through the remainder of the series of experiments.

The estimate of stairs climbed is a fairly accurate average, based on the extremely uniform round of places visited each day.

The record of hours devoted to sleep did not commence till July of that year. They vary from 8.20 to 8.35 hours in different months. I believe it probable that in periods I to VI the hours were not less than the latter figures, but in order to insure against an under-estimate of activities, I have calculated on the basis of 8.2 hours.

A rather problematical point as regards activity is the number of hours in the standing position, and the amount of activity of gentler type than would record on the pedometer. Much time was spent standing, as I made but little use of chairs in the laboratory. In order to insure against an under-estimate, the figure has been set at ten hours for periods I to VI inclusive. In the same spirit I have assumed an arbitrary figure of half the energy of an average day's walking to cover the undeterminable lighter activities. These data give us the following estimate of the daily energy output that would fulfil the ordinary expectation for this period:

	calories
Basal metabolism, 24 hours[5]	1724.0
Added for 5.8 hours sitting, $\frac{5.8}{24} \times 0.1 \times 1724$	41.7
Added for 10 hours standing, $\frac{10}{24} \times 0.2 \times 1724$	143.7
Added for walking 4.82 miles, $4.82 \times 0.6 \times 75.4$ (i.e., clothed weight)	218.1
Added for activities not shown on pedometer (estimated as 50 per cent of the walking)	109.0
Added for climbing 45 m. of stairs,[6] $45 \times 4 \times 75.4 \times 0.002343$	31.8

[5] Harris and Benedict's prediction for height, 181.2 cm.; weight, 71.81 kgm.

[6] Assuming a mechanical efficiency of 25 per cent.

Digitized by Google

Added for descending 45 m. of stairs[7]	15.9
	2284.2
"Digestion cost" of 3545 cal. food (6 per cent)[8]	212.7
Predictable calorie output	2496.9
Food eaten	3545.0
Deduction for excess feces (21 grams excess above the normal 30 grams) 21 × 6.2 cal	130.2
Net cal. from diet	3414.8
Discrepancy of intake over predictable need	+37 per cent

This diet seems, from the calculation, to be no less than 37 per cent in excess of the predictable need, while the diet in period II showed an excess of 21 per cent. But it is necessary to allow for the difference between a falling weight in period II and a rising weight in the later period. If we assume that the calorie value of the flesh gained and lost does not exceed that of pure fat, then the total oxidation of material (food and body fat) in period II lies 21 per cent to 23 per cent above the predictable figure, and the oxidation in period VI is between 27 per cent and 37 per cent above the prediction. *The discrepancy between the predictable and the observable expenditure has undergone an absolute increase and probably even a relative increase.*

Basal metabolism during over-nutrition. In June, 1917, the courtesy of Dr. F. G. Benedict and of Dr. T. M. Carpenter and their collaborators in the Carnegie Nutrition Laboratory supplied me with three determinations of my basal metabolism by their usual routine methods. On June 13 and 16 the tests were in the bed respiration chamber, Miss Corson in charge, at the Deaconess' Hospital, Boston, and on June 20 by means of the large Tissot spirometer and Haldane gas analysis methods, carried out by Doctor Carpenter. These tests were all of them on the high diet, the average intake of the three 2-day periods preceding these three tests being 3965 calories. As the experiments necessarily interrupted the diet, being taken at breakfast time on an empty stomach, and not being concluded for some hours, the average intake for the whole period of June 9 to 19 inclusive is only 3790 cal. On this diet of essentially 3965 calories representing the maximum intake up to that date, and at a body weight of 73.62 kgm., the recorded metabolism is very uniform with the single exception of the first respira-

[7] Assuming the descent to cost half as much as the ascent.

[8] Benedict and Carpenter (22).

Digitized by Google

tion period of the first day, the period of introduction to the apparatus, when the psychic effects undoubtedly militated against complete relaxation. Rejecting this half-hour period, the average of the other results, by indirect calorimetry from the gas analysis, are 73.32 cal. per hour, or 1762 cal. per day for the waking basal metabolism. The prediction for weight 73.6 kgm., height 181.2 cm. and age thirty-five, made by Harris and Benedict (19) is 1749 cal., constituting an almost perfect agreement with the finding.

A very high respiratory quotient should be noted; the figures being 0.94, 0.93 and 0.89 on the three different days. In the first two of these cases the non-protein respiratory quotient was 0.98. Thus even thirteen to fifteen hours after the last meal, and although fats were by no means excluded from the high carbohydrate diet, the oxidative processes were limited practically to carbohydrate and protein.

In spite of this fact that the high carbohydrate of the previous evening is still exerting a great influence upon the respiratory quotient, the metabolic rate conforms exactly to the prediction for the basal or post-absorbtive rate. The prolonged diet has *not raised the basal metabolic rate above the normal average.*

As I believe the figures have demonstrated that the metabolism as a whole is extravagant above the average, we shall have to look for the element of extravagance in some other factor than the basal rate.

Return to normal level. The return to a normal weight was accomplished in the summer months of 1917, while at the laboratories of the University of Illinois Medical School. During this period the food, urine and feces were analyzed for the determination of nitrogen balance. The urine analyses were continued most of the time to the end of the experiments on November 26, and the weighed diet up to that date was limited to the same set of foods as were used in the summer period. Thus the nitrogen exchange was followed in full or in part during the whole of these 4½ months. Table 3 summarizes these analytical data.

The diet differed from that of previous periods in the substitution of shredded wheat in place of the more difficulty analyzable white rolls, and also in the change from soft boiled eggs to a form of custard, which could easily be sampled for analysis when mixed and strained and ready to cook. Clear centrifuged butter fat was used in the summer months in place of commercial butter. Head rice, Quaker brand rolled oats and whole milk completed the diet. The analysis of the milk was by taking equal daily samples. The shredded wheat was

Digitized by Google

TABLE 3

Summary of nitrogen exchange

	PERIOD AND DATE												
	VII		VIII				IX			X	XI	XII	XIII
	1917 July 8–9	1917 July 10–14	1917 July 15–24	1917 July 25 to August 7	1917 August 8–29	1917 August 30 to September 1	1917 September 2–12	1917 September 13 to October 8	1917 October 9–12	1917 October 13–23	1917 October 24–27	1917 October 28 to November 10	1917 November 11–25
Duration in days	2	5	10	14	22	3	11	25	4	11	4	14	15
Dietary N.	20.26	19.74	15.68	13.60	13.42	13.50	13.37	14.60	15.13	15.71	15.88	15.05	15.97
Urinary N.		14.30	16.02	14.16	13.23			12.17		12.80	12.09	11.13	12.16
Fecal N.		3.54	1.59	1.27	1.02								*2.00
Daily N. balance		+1.89	−1.93	−1.82	−0.83								+ 1.81
N. balance for period		+9.47	−19.34	−25.53	−18.35								+27.07
			Loss in 46 days 63.2 gm.										
Calorie intake	4127	4110	2212	1811	1772	1788	2198	2537	2659				
Per day	av. 4115		av. 1874				av. 2441			2781	3183	2970	3204
Change of weight during period	+ 0.64		−9.58				−2.83			−0.55	+ 0.25	−0.57	−0.05
Change of weight per day	+0.091		−0.196				−0.069			−0.05	+0.063	−0.044	−0.003

* 12.01 grams in 6 days, November 14–19, inclusive.

Digitized by Google

broken small and mixed thoroughly in a large, moisture-proof container, from which the daily portions and the samples for analysis were taken. The rolled oats and the rice were similarly mixed and sampled. All these cereal samples were ground before dividing them into smaller portions for the analyses. The Kjeldahl-Gunning method was used in all the nitrogen determinations.

The first week of this summer series, on a diet of 4115 cal., represents very nearly the maximum capacity of the subject for continuous consumption, unless fats were to be used more freely. A substantial plus balance is shown both in nitrogen and in body weight. The period is too short to indicate the extent to which excess oxidation may have developed, but it is clear that the process had not gone far enough to prevent further fattening.

Following this was a period of low nutrition, lasting 7 weeks, with a daily average of 1874 cal. The intention was to dispose of the accumulated body fat without running into the condition of depressed basal metabolism that will occur when there has been a heavy loss of nitrogen. Accordingly the quota of eggs was somewhat increased, and the radical cut was confined to the cereal foods and the butter fat, the latter being entirely discontinued during most of the interval. It was impossible, however, to prevent a strongly negative balance of protein materials, so that the average daily weight loss of approximately 200 grams for the rest of the summer is accompanied by an average nitrogen loss of nearly 1.4 grams per day.

Final equilibrium. The attempts to reach a stable weight during September, October and November make it clear, first of all, that in spite of the considerable loss of nitrogen the body was by no means in a depressed metabolic state. It is impossible to tell whether the body was richer or poorer in protein constituents than it had been during the first equilibrium period (period II) of 1916. After more than a year of high calorie diet, with a fully adequate protein intake at all times, there can be no question that when the diet was changed in July the tissues were copiously stocked with protein materials. The figures obtained after July 15 can be extrapolated so as to show that between that date and the middle of September the body must have lost some 70 or 75 grams of nitrogen,—an amount that probably did not yet leave the body in any greatly depleted condition. When the diet was now increased to near 2500 calorie, this negative balance was checked, or possibly even changed to a small positive figure, but the loss of weight was not entirely overcome. The same was true in the

Digitized by Google

period on 2780 calories in October, the nitrogen balance being somewhat further improved, but the weight still continuing to decline. A virtually steady weight was at last established on an appreciably higher diet, from October 24 to November 25 inclusive, but not till the nitrogen balance had become definitely positive. Judging from the final fortnight, (period XIII), 3200 calories were now necessary to maintain a body weight of about 61.3 kgm.

During September, 1917, it was impossible to make a satisfactory record of activity, because at that time the exigencies of changing to a new residence caused a temporary increase of manual labor. The October and November records are free from such complications, excepting that the use of a bicycle was commenced at this point, to get to and from work. Habits of sitting and standing were about as in the early months of experimentation. The recorded activities are as follows:

	PERIOD AND DATE			
	X October 13–23	XI October 24–27	XII October 28 to November 10	XIII November 11–25
Duration in days	11	4	14	15
Sleep per day, hours	8.55	8.44	7.78	8.28
Walking per day, miles	4.76	7.62	3.58	4.35
Bicycle per day, miles	2.61	4 48	4.54	4.77
Stairs climbed per day (est.) meters	20	20	20	20

The bicycle route involved no steep grades, and always returned to the level of the original starting point. The bicycle was an exceedingly easy running one, capable of coasting freely on windless days on a gradient of 115 feet (35 m.) per mile, rider plus bicycle having a total weight of 83 kgm.

Without attempting to evaluate the energy used in bicycle riding, I believe it will be conceded to be insufficient to make the activities of the above periods appreciably greater than in periods II and VI. But the irregularities in sleep are probably a rather serious factor, and period XII is probably vitiated for purposes of comparison by the relatively poor "sleep" record. For this reason period XIII seems to be the principal one for a satisfactory comparison with period II, on the assumed basis that they represent essentially the same degree of muscle activity.

Digitized by Google

The summarized results of the last four periods are as follows:

PERIOD	DATES	INTAKE IN CALORIES	CHANGE OF WEIGHT PER DAY	REMARKS
			grams	
X	October 13–23	2781	−50	Maximum sleep. Activities below average
XI	October 24–27	3183	+63	Excellent sleep. Greater activities
XII	October 28–November 10	2970	−44	Imperfect sleep. Moderate activities
XIII	November 11–25	3204	− 3	Excellent sleep. Moderate activities

I place beside these for comparison the earlier period:

II	March 30–May 9, 1916	2744	−12	Excellent sleep. Moderate activities

In spite of the inescapable irregularities inherent in human experimentation of this sort, a comparison of the forty-one days in 1916 with the final thirty-three days, both of which were at practically

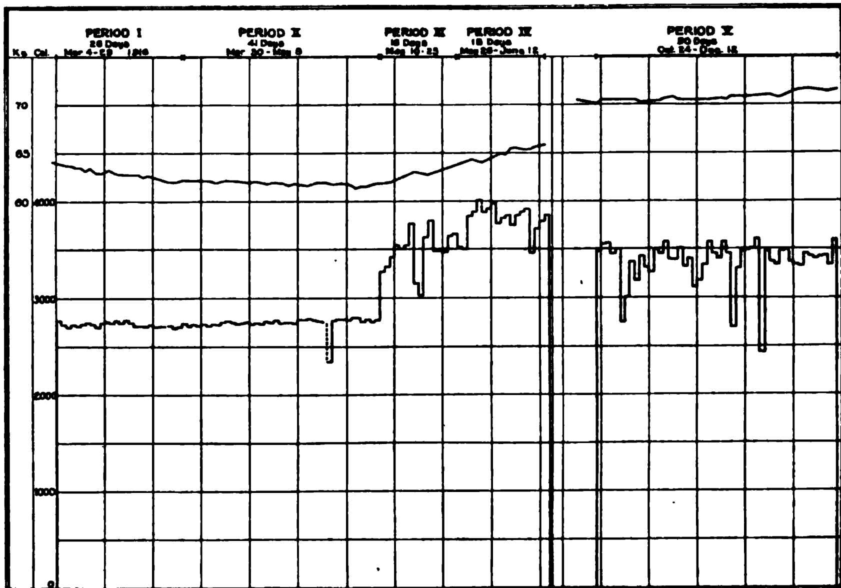


Chart of food intake and body weight, 1916

Digitized by Google

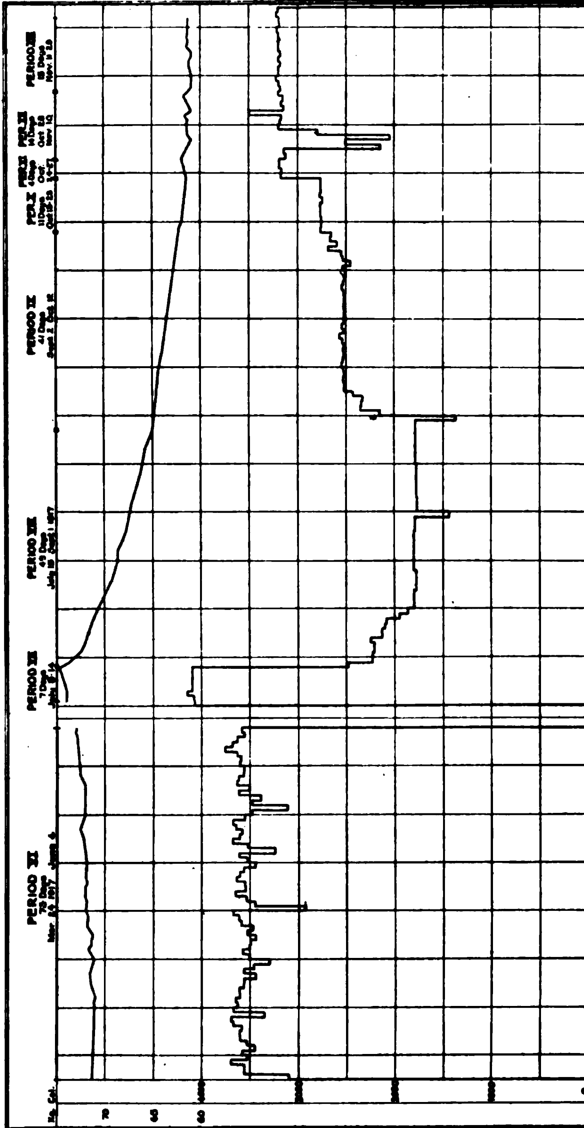


Chart of food intake and body weight, 1917

identical average body weights, brings out very definite evidence of a marked increase in the daily caloric requirement. It is not safe to attempt to calculate exactly how great this change has been, but on the face of the results from the final fifteen days, the daily demand seems to have risen by some 300 to 400 calories.

DISCUSSION

The general results of these experiments are that this example of a person belonging to the difficultly fattening type was found to show a wasteful rate of oxidation during all the feeding experiments, including both the periods in which the diet was moderate or low and those in which a large excess of starch was superposed upon the normal diet. During the prolonged periods of high diet this wasteful oxidation became more pronounced, and it continued so throughout the following periods of under-nutrition, so that even after the body had been brought down again to its original weight, it required more food to keep it at steady weight than had been necessary at the start.

In a preliminary report of these experiments that was made in 1917 (25) considerable uncertainty was expressed as to whether or not these figures could be used as evidence for a compensatory excess oxidation during high feeding. This doubt was based on the fact that a comparison between the nutritive exchange during high feeding and that during the final low-weight periods could only lead to inconclusive results. In the present paper the final low-weight periods are not used for this comparison, and the extravagance of the calorie exchange during over-nutrition is appraised by comparing it with the initial determination of minimum need, as made in 1916. It is believed that this plan of analysis is justified; firstly, because the calorie demand in the final periods is so far above the need as found in 1916 that it seems to show a hangover effect from the months of excess nutrition; and, secondly, because even the initial need is noticeably in excess of the expectation by dead reckoning.

The basal rate of metabolism as determined in a reclining position before breakfast did not rise above the average expectation for the subject's age, weight and height. Pulse and blood pressure were also entirely normal.

It seems clear that throughout the entire experimental series there was some factor at work which caused fuel food to be burned more freely than in the average individual. This factor was not an overactive thyroid as attested by the entirely normal basal metabolism.

Digitized by Google

It is possible that a part of the waste is attributable to neuromuscular factors. During all the experimental periods the greater part of the daily activities were of the less intense variety, the calorific cost of which is always problematical, because it can never be predicted how much will be wasted in the increased tone of the unemployed muscles. This undeterminable expenditure may easily have varied from the average expectation, and may be responsible for some of the unexplained energy expenditure. But if this were the whole explanation, we ought to find a lessened wastefulness and not an increase during the months of over-feeding when there was a continued stuffed feeling and a disinclination to exertion. For this reason it seems more probable that the main factor is not to be sought in neuromuscular habits, but in some factor in the chemistry of nutrition.

The nitrogen balance may very easily be connected in some way with this problem, for although the actual intake of proteins was never abnormally high, the liberal calorie allowance of the experimental diets and of the subject's previous dietary habits was very favorable to an accumulation of nitrogen. Even the last tests, after there had been a loss of 70 grams or more of nitrogen, may have been under the influence of superabundant stores of protein materials, as that 70 grams were only removed after a maximum storage must have been attained, and by the time that the last experimental weeks had been reached, some of the lost nitrogen had been restored. If the factor causing extravagance is related to this supposed nitrogen enrichment, it is not to be compared with the plethora effect observed by Atkinson and Lusk (5), but it may very possibly be comparable to the "secondary effect" of protein enrichment, which according to Rubner (1) can raise the specific dynamic effect of the food without raising the basal rate. The present experiment differs from Rubner's in that the food for which the specific dynamic effect must be augmented is largely starch instead of protein.

It is also possible that nitrogen enrichment may not be the major explanation of the nutritive condition. For there is still the alternative that von Noorden's (15) suggestion respecting the obese type may have its converse, and the spare type be accounted for by any factor that produces a high "cost of digestion," just as the obese may be supposed to suffer from an abnormally low "cost of digestion." The decision between these alternatives would only be possible after an extension of the tests beyond the limits that have been practicable in the present investigation.

Digitized by Google

SUMMARY

1. During periods aggregating about three hundred and seventy days on experimental diet, a person of the difficultly fattening type was investigated, first, to determine the minimum food required for maintenance of weight at the customary level; second, to ascertain whether and to what extent an excess of starchy food would be stored by this type of person as adipose in a long period of superabundant measured diet; and third, to ascertain whether after the body was returned to the initial weight with least possible loss of nitrogen, any change had occurred in the minimum requirement of food.

2. The person was found to owe his resistance against fattening to an extravagant calorie requirement which persisted at all times, despite a moderate daily round of activities.

3. This extravagance increased during the course of the excessive carbohydrate diet, and stayed above the initial level even after the return to normal weight.

4. The basal metabolic rate was not involved, but remained strictly normal.

5. The high calorie output and consequent resistance against fattening may find its explanation either in a condition of nitrogen enrichment, or in an upward variation of the "cost of digestion" (and assimilation) of starchy food.

Grateful acknowledgments are due to the Nutrition Laboratory of the Carnegie Institution, to Dr. F. G. Benedict, in charge of that laboratory, to Miss Corson, at the Deaconess' Hospital, Boston, and particularly to Dr. Thorne M. Carpenter for active interest in the determination of basal metabolic rate in June, 1917; and to the University of Illinois Medical School, Laboratory of Physiological Chemistry, Chicago, where I received liberal backing for the portions of the work done in July and August, 1917, through the generous recommendation of Dr. W. H. Welker. All other parts of the experiments here reported are from the Laboratory of Physiological Chemistry, Department of Physiology, University of Missouri.

BIBLIOGRAPHY

(1) Rubner: Gesetze des Energieverbrauchs, 1902.

(2) Dengler and Meyer: Zentrbl. f. d. gesammt. Physiol. u. Path. d. Stoffwechsels., 1906, 288.

(3) Müller: Zentrbl. f. d. gesammt. Physiol. u. Path. d. Stoffwechsels., 1911, 617.

Digitized by Google

(4) Grafe and Koch: Deutsch. Arch. f. Klin. Med., 1912, cvi, 564.
(5) Atkinson and Lusk: Journ. Biol. Chem., 1919, xli, p. xiii.
(6) Anderson and Lusk: Journ. Biol. Chem., 1917, xxxii, 421.
(7) Loewy and Zuntz: Berl. Klin. Wochenschr., 1916, 825.
(8) Zuntz and Loewy: Biochem. Zeitschr., 1918, xc, 244.
(9) Benedict, Miles, Roth and Smith: Carnegie Instit. Pub. 280, 1919.
(10) Joffe, Poulton and Ryffel: Quart. Journ. Med. (Oxford), 1918, xii, 334.
(11) Neumann: Arch. f. Hyg., 1902, xlv, 1.
(12) Grafe and Graham: Zeitschr. f. physiol. Chem., 1911, lxxiii, 1.
(13) Rubner: Beiträge zur Ernährung im Knabenalter mit besonderer Berücksichtigung der Fettsucht, Berlin, 1902.
(14) Magnus-Levy: Zeitschr. f. Klin. Med., 1906, lx, also xxxiii, and other references.
(15) von Noorden: Metabolism and practical medicine; see discussion on p. 693–715, esp. p. 698; also full literature references on obesity.
(16) Means: Journ. Med. Research, 1915, xxvii, 121.
(17) Gebhart and DuBois: Archives of Int. Med., 1916, xvii.
(18) Atwater and Woods: U. S. Dept. Agric., O. E. S. Bull. no. 28, 1896.
(19) Harris and Benedict: Carnegie Instit. Pub. no. 279, 1919.
(20) Benedict and Murschhauser: Carnegie Instit. Pub. no. 231, 1915.
(21) Benedict and Cathcart: Carnegie Instit. Pub. no. 187, 1913.
(22) Benedict and Carpenter: Carnegie Instit. Pub. no. 261, 1918.
(23) Bischoff and von Voit: Gesetze der Ernährung des Fleischfressers, 1860.
(24) von Voit: Handb. d. Physiol. d. allg. Stoffwechsels, (Vol. vi, pt. 1, of Hermann's Handbuch), 1881.
(25) Gulick: This Journal, 1918, xlv, 549.

Digitized by Google

Digitized by Google

／# THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 60 MAY 1, 1922 No. 3

STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

I. Effect of the Various Factors Composing a Tropical Climate on Growth and Fertility of Mice

E. S. SUNDSTROEM

From the Laboratories of Biochemistry, University of California, Berkeley

Received for publication January 3, 1922

As a preparation for work on the acclimatization of man to a tropical environment I conducted in 1919–20 a number of experiments on the physiological behavior of mice in an artificially produced tropical climate. I hoped by these experiments to gain an insight into the mechanisms of adaptation that are released by transfer to a hot environment. During the course of my investigation and more so after finding an opportunity to continue my work under conditions actually prevailing in the tropics, I have become convinced that a considerable part of research in the climatological physiology of hot countries may advantageously be done in temperate climates. By experiments on animals a broad foundation could be built for this science. When results primarily applicable to man are desired it becomes, naturally, necessary to confirm these results by anthropological observations in the Tropics. When these latter, however, are conducted alone, the difficulty in finding homogeneous material and the lack of synchronous controls will render them difficult to interpret. The ideal procedure in work along these lines is a coöperation of anthropological observation and animal experiment.

So far as I am aware, there does not exist at the present time any investigation on a large scale on animals which have been exposed to external heat for several generations with the physiological effects arising therefrom in view. A considerable number of observations

Digitized by Google

is reported on the effect of exposures to heat of short duration. For solving certain well-defined physiological problems such methods are adequate. Very little of the information so acquired can, however, be applied to the problems connected with the acclimatization of the white race to a tropical climate. Incomparably more may be learned from series extending for several generations. Longer series have been reported by Przibram (1) on rats and by Sumner (2) on mice but both these investigators have not been concerned about the physiological aspects of the problem. Both have discovered very interesting morphological changes arising from exposure to heat which still await their physiological explanation. Recently Steinach and Kammerer (3) have taken a step in the outlined direction, but their investigation is more especially concerned with the sexual functions. Their material on growth is limited to weighings of the members of *one* litter and is, naturally, entirely inadequate for the purpose.

For the physiological interpretation of environmental experiments it is desirable to maintain as closely as possible a fixed difference in temperature between the hot series and the controls. Sumner paid very little attention to this prerequisite. The difference in temperature between his cold and warm room series was very variable and reached sometimes 18°C. No attention was paid to the humidity of the air. The temperature of the cold room was probably below the optimal range for the physical welfare of mice, which would explain the fact that Sumner failed to discover any appreciable difference in growth between his heat mice and his controls. On the other hand, the Vienna investigators have in their experiments adopted the incubator principle and kept their rats at constant temperature and humidity. As considerable variations may occur in an actual tropical climate and as it has, furthermore, been shown that mice thrive better when the temperature is slightly variable (4) I have in my experiments avoided constant temperatures, but at the same time insisted upon a constant interval between the temperatures in the hot and temperate room.

I was fortunate in securing breeding mice for my investigation from a stock that for five years had been promiscuously bred and thoroughly observed by Professor Robertson and later by Doctor Hagedoorn. The former has published extensive data on the normal growth and fertility of these mice (5). His work has served me as a model. My thanks are due for personal advice and for the encouragement Doctor Robertson has given me. I am also indebted to Doctor Hagedoorn for many suggestions in connection with my work.

Digitized by Google

Robertson (6) has furnished statistical evidence to show that the variability of his mouse stock decreased progressively. Since the variability of weight of my control animals, as I hope to show in this paper, was still smaller than in Robertson's 1916 series, I venture to maintain that the genetical homogeneity of my mice, at least as far as their size is concerned, was as complete as can be wished for in work of this sort. This homogeneity was further enhanced by the precaution I took, in making up various series, to divide equally a number of litters among them. The total number of animals was in the neighborhood of one thousand. Approximately half of this number were employed for growth studies. The rest were used for various other experiments, the results of which will be reported in subsequent papers.

Although my principal object in view was to compare the growth of mice that either had been brought into the hot room at an early age—as a rule when 3 weeks old—or which were born in the same environment, with the growth of mice that were continuously kept at ordinary room temperature, I added to my program a number of other series which I hoped might elucidate the rôle such climatic factors as light and circulation of air play in the adaptation to a tropical climate. In addition to the control series that was reared in subdued light I kept in the "temperate" room two other series, "immigrants" and "descendants" (when these terms are used in my papers they mean mice that were transferred to the new environment when 3 weeks old and mice that were born there, respectively) in which the animals were exposed to the radiation of strong artificial light. In the hot room two corresponding series of "light" mice were kept. The effect of air in motion was studied only in the hot room and in subdued light. The "hot, still air, subdued light, descendant" series was divided in three subseries corresponding to four succeeding generations of mice. The second of these subseries consisted of mice from the second and third generations, whose growth curves were identical and which, because of small absolute numbers, have been considered together.

The "tropical" room had an air capacity of 6½ cu. m. which is approximately the same as the size of the rooms the Vienna biologists employed for their heat experiments. It was fitted with double walls, which were separated by an air space. It further had a double door and a double window. The inner wall and the inner door were covered with heat insulating material. Although no draught could be detected, the slow passage of air through the walls seemed to be sufficient for ventilation. No appreciable rise of carbon dioxide was found, which probably was

Digitized by Google

due to the comparatively small number of mice that simultaneously occupied the room.

The heating of the room was accomplished by an electric hot plate, the heat of which could be regulated according to the outside temperature. By exercising some care in observing the temperature changes, especially in summer, I succeeded in keeping the dry bulb temperature of the hot room close to 10°C. above the temperature of the room where the control series were kept. This latter was steam heated in winter. The high humidity of the "tropical" room was in the beginning maintained by a dropping device, drops of distilled water of regulated size falling direct on the hot plate. Later it was found that a large basin filled with water and placed on the hot plate served the same purpose.

The table below embodies observations of the dry and wet bulb readings taken continuously in January and February, 1919.

	DRY BULB TEMPERATURE			WET BULB TEMPERATURE		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Temperate room						
a.m.	22.7	13.0	18.3	16.8	9.3	13.5
p.m.	26.8	21.1	23.7	20.6	16.0	18.2
Average	24.8	17.1	21.0	18.7	12.7	15.9
Hot room						
a.m.	31.5	23.8	29.2	29.7	19.8	25.3
p.m.	38.0	29.4	34.4	33.0	26.0	30.4
Average	34.8	26.6	31.8	31.4	22.9	27.9

Another series of observations taken in 1920 rendered the same dry bulb averages and ranges but a slightly lower humidity. Thermograph records were taken for long periods of time and the results that were calculated from them confirmed the figures obtained from dry bulb observations in the accurate series.

The source of light in the experiments consisted of "Mazda" globes. Two 60 watt lamps were employed in the temperate room and a 100 watt one in the hot room. The globes were suspended about 50 cm. above the bottom of the cages. The light was turned on in the morning and off at night. I admit that my imitation of the tropical sun was far from ideal. I believe, however, that the employment of an arc lamp or other light of a spectral composition that would more closely

Digitized by Google

have resembled sunlight would, for albino mice, have been contraindicated. I am inclined to the view that the amount of light that penetrated into deeper tissues was as large in these experiments on mice as may occur in man when exposed to the tropical sun. It has been found that tungsten light contains an appreciable percentage of shorter waves. The incipient pigmentation that was observed in a number of "light" mice which will be reported in another paper of this series, and further the indisputable effect of the light on the growth curves of the mice, bear evidence that the light radiation *per se* was capable of producing physiological effects. The different methods the mice invented to protect themselves further proves that they felt strongly the impact of the light. It was, e.g., a common sight to see all the mice in a cage side by side outstretched on their backs with the feet across their bellies.

The ventilating system for the "wind" experiments consisted of an electric wall fan, about 30 cm. in diameter, suspended in horizontal position 25 cm. above the bottom of the cages. The lower end of its case was fitted into a hole in the top of a wooden box, one side of which was removed. The air entered through a cylindrical paste board extension of the fan case that almost reached the ceiling of the room. The air was then blown through the open side of the box in a direction that was opposite to the place where the "still air" cages were kept. A circular air current was thus established. By repeated tests I found that other parts of the room were protected from the draught.

As mouse cages I employed grey enameled wash basins, 11 inches in diameter, which were covered with a wire net, three meshes to an inch. The bottom of the cage was covered with sawdust, which was changed at frequent intervals. In the "light" series the food was put into the cage, in others it was placed on top of the wire net. In the majority of cages about half a dozen animals were confined in each cage. Double this number was sometimes kept in the "light" and "wind" cages without any bad effects on the inmates. In most of these series a stimulation of growth was evident, which would hardly have occurred if any impairment of their physical welfare had resulted from overcrowding.

The food of the mice was strictly homogeneous throughout the investigation and the same for all series. It consisted chiefly of a mush that was freshly prepared every morning of yellow corn meal, 6 parts; rice, 2 parts; rolled barley, 2 parts, and powdered meat scraps, 1 part. The mush was given in the forenoon. In the afternoon a handful of fresh rolled barley was strewn into each cage. Greens were supplied once or twice a week. All the mice had access to water, which was supplied

Digitized by Google

by the drop-tube method. The normal growth of the control series seems to prove that the mice were properly fed. To insure this I further practised the rule of giving them a surplus of food in excess of their consumption.

The general health of the mice was very good; no cases of infectious diseases occurred in the mouse colony. All the deaths were, as far as I could ascertain, caused either by malnutrition in newly-born mice or by accidental causes such as wounds received in fighting or overheating in older mice. A parasitological survey of a number of feces and intestinal contents revealed nothing exceptional.

The young mice were separated from their mothers when they were 3 weeks old. Since it proved impossible to obtain a sufficient number of "descendant" mice in the hot room from conceptions taking place there, I was compelled to introduce a number of females in a pregnant state in order to fill my "descendant" series.

Because of the meager information available on the effect of the climatic environment on the velocity of growth it has been customary to attribute differences in physical development to nutritional and in man also to sociological factors (7). Certain facts seem to indicate, however, that the climate may play an important rôle in this respect. It has been found in cooler climates that the principal part of the growth of children takes place during summer (8). In some as yet unpublished investigations on the growth of children of Scandinavian parentage in California I observed an acceleration of the third growth cycle in comparison with children from well-to-do classes in Scandinavia in which no undernutrition can be suspected to occur. Zoölogists have further presented evidence to show that species of animals that inhabit a wide area decrease in size toward the equator (9).

As known, the third growth cycle coincides with the maturing of the sexual organs. The time of the first menstruation in females forms a convenient measure of this event. It has been anthropologically and experimentally proven by Steinach and Kammerer (3) that an increase in external temperature stimulates the endocrinal mechanism that regulates the sexual development. These authors have further demonstrated that while a stimulation may take place up to a certain point, beyond this point a retardation may set in. Stefansson (10) has recently added valuable observations that confirm these conceptions. He points, namely, to the fact that menstruation commences in Eskimo girls at an age of 10 to 13 years and attributes this to the tropical temperature that is maintained in the Eskimo huts.

Digitized by Google

When the heat is combined with high humidity it may be anticipated that the stimulation due to temperature may be counterbalanced by factors working in an opposite direction, primarily by the checking effect the tropical climate has on the output of heat from the body. Sufficient evidence exists to show that several mechanisms may be operating in the interest of the organism to adjust the disproportion between heat production and heat output. The chemical heat regulation, undoubtedly, in small animals plays an important rôle in this respect (11). Some observers contend that the basal metabolism even of man may be diminished in the Tropics (12). The same purpose may be attained by an enlargement of the cooling surface. In the next paper the possibility of modifications of the body capable of producing a larger skin area will be discussed at length. In this connection it suffices to point out that a suppression of growth would answer the same purpose, rendering the body area relatively larger.

Robertson (13) has discussed the statistical methods that enable the biologist to decide whether the number of animals employed is sufficient for the purpose in view. If an accuracy of 1 per cent is required the formula reads

$$N = \sqrt{\frac{100 \times 0.6745 \times \sigma}{M}},$$

where N is the number of variates sought, M the mean and σ the standard deviation. Only a few of my weight series reach this high degree of accuracy. I have therefore deemed it necessary, when comparing the growth curves, to express the difference between each pair of them in terms of the probable error of the same difference. These multiples of the probable errors have been plotted as curves.

The mice were weighed at weekly intervals, in the forenoon before feeding. The weights were recorded to the nearest tenth of a gram. My growth studies cover the principal part of the growth period up to 20 weeks of age. A part of the material was weighed, however, only until they were 12 or 13 weeks old. The growth curves for the animals that were weighed above this age form a direct continuation of the curves for the larger material below the same age, which probably is due to the homogeneity of my mouse colony.

The results of my growth studies are collected in tables 1 to 4. Tables 1 and 2 contain the average weights for each week from the third to the twentieth. Tables 3 and 4 contain data on the variability of the body weights.

Digitized by Google

The data from tables 1 and 2 are plotted in two graphs, no. 1 for male and no. 2 for female mice. These curves obviously do not require any explanation. Figures 3, 4 and 5 give in form of curves all the data required to add statistical weight to the averages. These data have

TABLE 1

Average weight of male mice

	TEMPERATE ROOM			HOT ROOM						
		Light		Stagnant air						Wind
				Subdued light				Light		
					Descendants					
	Controls	Immigrants	Descendants	Immi-grants	1 genera-tion	2 + 3 genera-tions	4 genera-tions	Immi-grants	Descend-ants	
Number weighed	32	24	26	43	56	22	14	11	11	16
age, weeks										
3	6.6	6.0	8.1	7.3	7.4	6.7	6.2	6.3	5.6	7.4
4	9.4	9.7	11.9	9.7	10.1	8.7	8.5	8.4	7.5	9.5
5	12.3	13.0	15.7	11.9	12.0	11.0	12.3	9.1	10.0	11.8
6	14.4	16.4	18.2	13.0	15.1	12.3	14.6	10.8	10.9	13.7
7	16.7	18.2	20.6	13.8	17.2	14.2	16.2		12.8	15.0
8	18.9	19.5	21.9	16.0	18.6	15.8			15.0	17.6
9	20.7	20.3	22.7	17.5	19.9	16.5			17.1	19.1
10	20.7	20.9	23.1	19.0	20.8	17.4			17.2	20.2
11	21.2	21.6	23.5	19.4	20.8	18.2			18.4	22.4
12	21.7	22.0	23.9	20.2	21.9	19.2				22.6
Number weighed	12	21	17	14	19					11
age, weeks										
13	22.1	22.5	24.2	21.2	21.4					22.1
14	23.5	22.8	24.2	21.1	20.9					22.7
15	23.8	23.5	24.6	21.3	22.0					23.2
16	23.8	23.9	25.2	21.7	22.6					23.1
17	24.2	24.1	25.4	22.0	22.1					23.2
18	24.0	23.9	26.1	22.2	22.2					23.4
19	24.2	24.1	24.9	22.3						23.8
20	24.3	25.5	25.6	22.5						25.1

already been explained as consisting of multiples of the probable error of the difference between the averages. The curves have been drawn with the age—in weeks—plotted on the abscissa and the multiples of the probable error on the ordinates. The position of the curve above the

Digitized by Google

abscissa indicates a stimulation of growth in comparison with the series of mice that in the special case has been accepted as control, a position below the abscissa means retardation of growth. For a single curve a deviation of three to four times the probable error is desirable in order

TABLE 2

Average weight of female mice

	TEMPERATE ROOM			HOT ROOM						
		Light		Stagnant air						Wind
				Subdued light				Light		
					Descendants					
	Controls	Immigrants	Descendants	Immigrants	1 generation	2 + 3 generations	4 generations	Immigrants	Descendants	
Number weighed	34	10	27	45	36	24	11	23	24	14
age, weeks										
3	6.3	6.3	7.6	7.5	6.7	5.9	6.0	6.8	6.4	7.7
4	9.7	10.6	11.1	9.7	9.5	7.7	8.5	9.5	8.5	9.4
5	12.4	13.7	14.5	11.7	12.1	9.6	12.4	10.6	10.4	11.7
6	14.4	15.1	15.8	13.1	13.9	10.9	13.4	11.2	11.5	13.0
7	15.9	16.7	17.1	14.5	15.1	12.0	14.8	12.6	12.0	14.2
8	17.4	17.5	17.9	15.9	16.0	13.0		14.2	14.2	15.4
9	18.5	18.9	18.9	16.6	17.7	13.6		15.2	15.4	16.7
10	19.0	18.9	19.6	17.2	17.8	14.8		15.6	16.4	17.9
11	19.8	19.4	19.8	17.9	17.9	16.2		16.4	17.5	18.5
12	20.2	20.0	20.3	18.5	18.0	15.9		16.9	18.0	19.4
Number weighed	14	7	16	17	19					10
age, weeks										
13	20.6	20.6	20.9	18.4	18.3			17.1	17.9	18.3
14	21.1	20.4	20.7	18.1	18.2					19.6
15	20.9	21.0	21.7	18.7	17.8					18.5
16	21.0	21.6	21.3	18.6	19.4					19.7
17	21.6	22.2	22.1	18.4	18.1					19.4
18	21.8	22.1	22.3	18.1	17.9					19.6
19	22.4	22.7	21.3	18.5						20.1
20	22.7	22.5	22.9	20.0						20.6

to insure that very small chances exist that the observed difference is not a true one. These chances are for three times the probable error 1:21 and for four times the same error 1:142. If, however, the male and the female curves are both located on the same side of the abscissa

Digitized by Google

smaller deviations of these curves may be significant as adding a further weight to the averages.

In figure 3 the growths of the two "temperate, light" series and the "hot, stagnant air, subdued light, immigrant" series have been compared with the "temperate, subdued light" series as control. In figure 4 the "hot, stagnant air, subdued light, immigrant" series serves as a

TABLE 3

Variability of weight in male mice

AGE	TEMPERATE ROOM			HOT ROOM				
		Light		Stagnant air				
				Subdued light				
					Descendants			
	Controls	Immigrants	Descendants	Immigrants	1 generation	2 + 3 generations	Light descendants	Wind
weeks								
3	23.2	(11.7)	21.1	23.7	29.5	28.8	18.4	17.9
4	22.3	19.5	19.5	19.4	22.6	23.2	19.6	20.8
5	19.4	15.4	12.5	20.9	19.2	20.5	15.9	19.7
6	20.3	9.8	11.5	21.9	18.9	19.1	17.8	21.2
7	19.1	9.2	9.3	23.8	19.2	14.2	17.7	21.5
8	14.2	7.4	7.8	20.7	17.0	16.0	17.7	20.1
9	10.2	7.5	7.7	17.2	13.6	14.7	14.0	16.6
10	9.2	7.1	8.0	16.3	13.6	12.1	12.3	13.4
11	8.0	6.7	6.2	15.0	12.9	16.8	10.2	11.8
12	7.1	7.2	7.1	14.0	11.6			11.8
13	7.7	7.3	7.1	13.2	10.0			11.0
14	7.7	7.5	7.9	9.1	11.2			10.7
15	8.2	7.6	8.0	9.5	9.4			10.6
16	7.9	8.8	8.0	9.7	11.4			9.8
17	7.6	9.3	6.5	9.2	11.3			11.0
18	8.3	9.1	7.7	7.0	10.4			13.0
19	(4.7)	9.7	7.1	6.8				12.0
20	(4.9)	9.4	8.2	(5.7)				10.4

base line and the curves correspond to the "hot descendant" series of the first and later generations and to the "hot, wind" series. Finally, in figure 5 three pairs of various "descendant" series are compared with the corresponding "immigrant" series as base line.

The material at our disposal does not seem to leave any doubt that the radiation of light acted as a stimulant to the growth of those mice

Digitized by Google

that were exposed to light at ordinary room temperature. This applies particularly to those mice which were born in that specified environment. The difference in weight between the "light" and control series seems to diminish with increasing age. It is impossible to decide to what extent the effect of light on growth may be due to the heat rays and how much to rays of a short wave length. The possibility that, e.g.,

TABLE 4

Variability of weight in female mice

AGE	TEMPERATE ROOM			HOT ROOM					
	Controls	Light		Stagnant air					Wind
				Subdued light			Light		
		Immigrants	Descendants	Immigrants	Descendants: 1 generation	Descendants: 2 + 3 generations	Immigrants	Descendants	
weeks									
3	24.3		21.7	26.3	30.0	20.3	21.0	17.5	21.7
4	20.1	12.5	18.3	22.9	24.1	16.8	21.8	15.9	24.9
5	18.1	10.3	14.8	21.4	17.8	17.0	18.1	10.9	24.9
6	16.2	10.5	11.4	20.6	17.6	17.1	13.5	13.3	28.3
7	13.3	6.3	10.8	18.1	15.3	18.5	18.3	14.3	28.6
8	12.6	6.5	8.7	16.7	14.5	18.4	14.4	11.4	25.2
9	11.4	8.9	9.3	16.2	13.0	18.5	12.5	9.7	19.3
10	11.4	7.0	9.0	15.2	11.9	18.7	15.1	7.7	17.6
11	10.1	7.5	7.4	15.8	13.1	17.2	13.2	8.6	16.0
12	10.4	7.7	7.5	15.7	13.2	17.0	13.4	7.7	15.6
13	10.6	7.1	7.3	14.4	14.0		12.0	7.7	(21.2)
14	9.4	7.5	8.8	(21.2)	14.1				11.7
15	10.9	6.9	7.1	15.2	12.4				18.2
16	10.1	6.6	7.4	15.4	11.9				8.5
17	7.4	6.8	5.0	16.3	12.2				9.5
18	7.4	7.0	8.6	15.4	10.6				10 2
19	7.8		5.6	16.7					10.5
20	(9.3)		9.4	15.9					10.9

a greater amount of exercise of the "light" animals may have contributed to the stimulation of growth can be excluded. It was found that these mice rested for at least as long periods of time as the controls.

The physical development of mice that, when 3 weeks old, were transferred to the "tropical" room and there kept in a stagnant hot atmosphere, was uniformly retarded. These experimental results are

Digitized by Google

in full agreement with what might have been expected. Mice that are born in the hot environment differ from the "immigrants" in that their growth curve in its earlier part is at a considerably higher level and

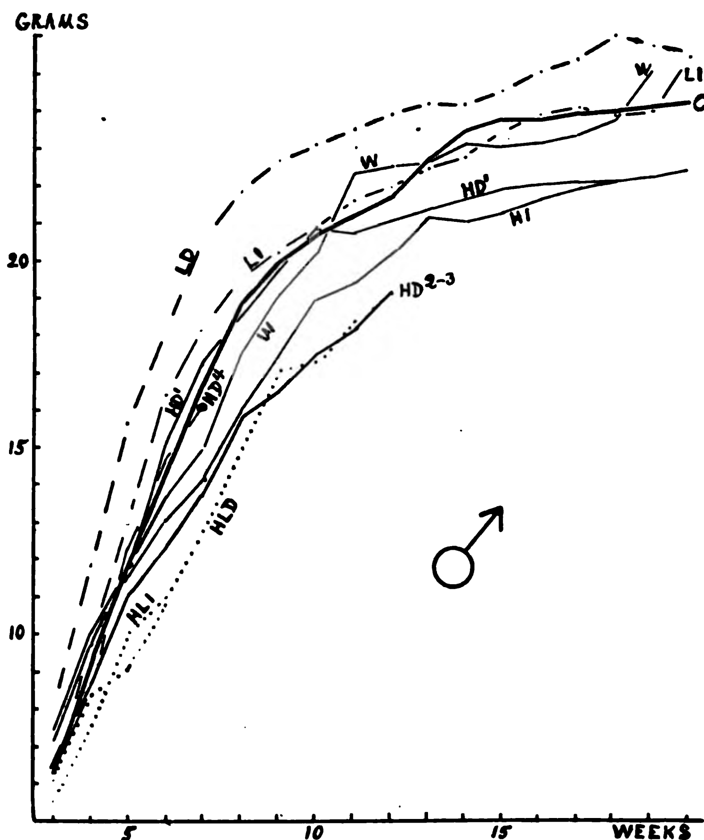


Fig. 1. Comparison of growth of male mice. Average weights. C = Controls; LI = light, immigrants; LD = light, descendants; HI = heat, stagnant air, subdued light, immigrants; HD^1 = heat, stagnant air, subdued light, descendants, 1st generation; HD^{2+3} = heat, stagnant air, subdued light, descendants, 2nd and 3rd generation; HD^4 = heat, stagnant air, subdued light, descendants, 4th generation; W = heat, wind; HLI = heat, stagnant air, light, immigrants; HLD = heat, stagnant air, light, descendants.

coincides with the control curve at least as far as the males are concerned. At a more advanced age the "descendants" seem to cease to grow until their curve coincides with the "immigrant" curve.

Digitized by Google

In the next two generations of mice born in the hot room we miss the initial stage of comparatively rapid growth and the whole curve remains low, especially for the females. This would possibly indicate that in these generations the unfavorable effect of the new environ-

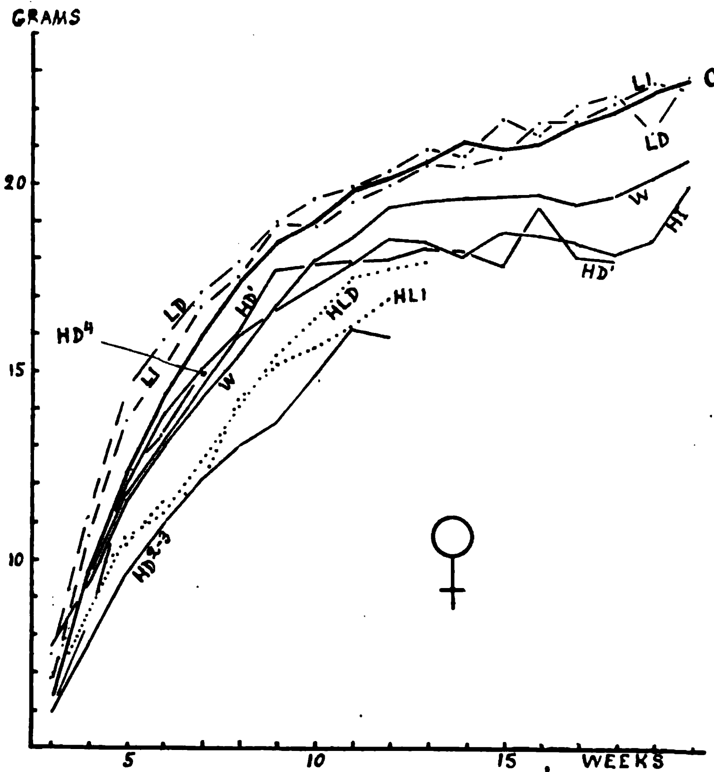


Fig. 2. Comparison of growth curves of female mice. Average weights. C = Controls; LI = light, immigrants; LD = light, descendants; HI = heat, stagnant air, subdued light, immigrants; HD^1 = heat, stagnant air, subdued light, descendants, 1st generation; HD^{2+3} = heat, stagnant air, subdued light, descendants, 2nd and 3rd generations; HD^4 = heat, stagnant air, subdued light, descendants, 4th generation; W = heat, wind; HLI = heat, stagnant air, light, immigrants; HLD = heat, stagnant air, light, descendants.

ment becomes manifest. It is regrettable that the study of the fourth generation was continued only until the seventh week. The considerable rise in the growth curve of this generation may signify that at this point of racial adaptation the adjustment of the energy balance

Digitized by Google

had been taken over by other efficient mechanisms so as to allow the growth to proceed at a velocity that approaches the normal.

We have seen that radiation of light stimulates physical development when the environmental conditions otherwise are favorable for a normal

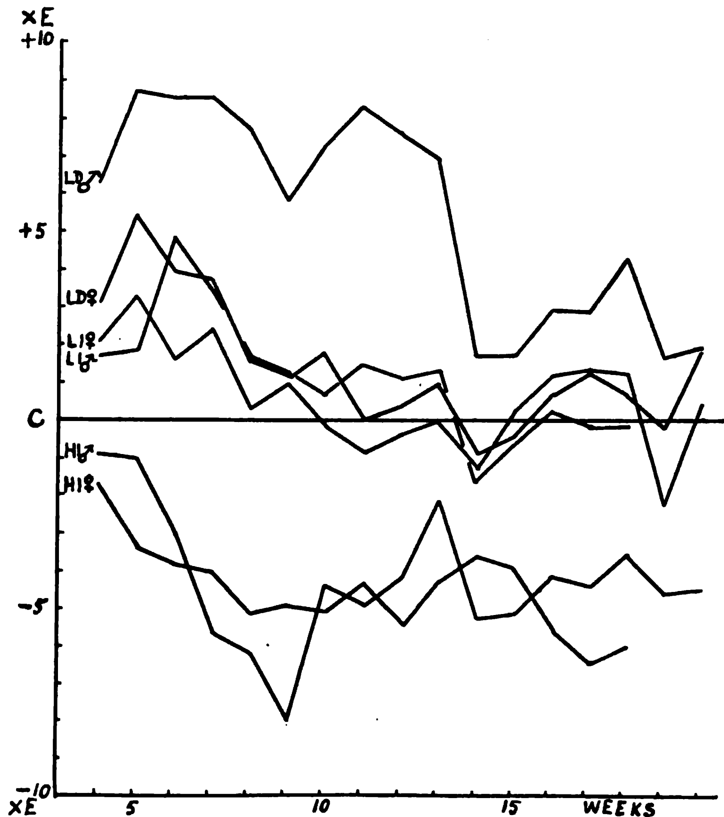


Fig. 3. Comparison of growth curves of "light" and "heat, immigrant" mice with control mice. Multiples of probable error of difference, *E*, between average weights. *C* = Controls; *LI* ♂ = light, immigrants, males; *LI* ♀ = light, immigrants, females; *LD*♂ = light, descendants, males; *LD* ♀ = light, descendants, females; *HI* ♂ = heat, stagnant air, subdued light, immigrants, males; *HD* ♀ = heat, stagnant air, subdued light, immigrants, females.

output of heat. When these conditions are not present, e.g., when the animals are confined to a hot room with stagnant air the disproportion between heat production and heat output becomes so great that

Digitized by Google

the organism reacts with retardation of growth in a degree that exceeds the retardation in heat without the addition of light.

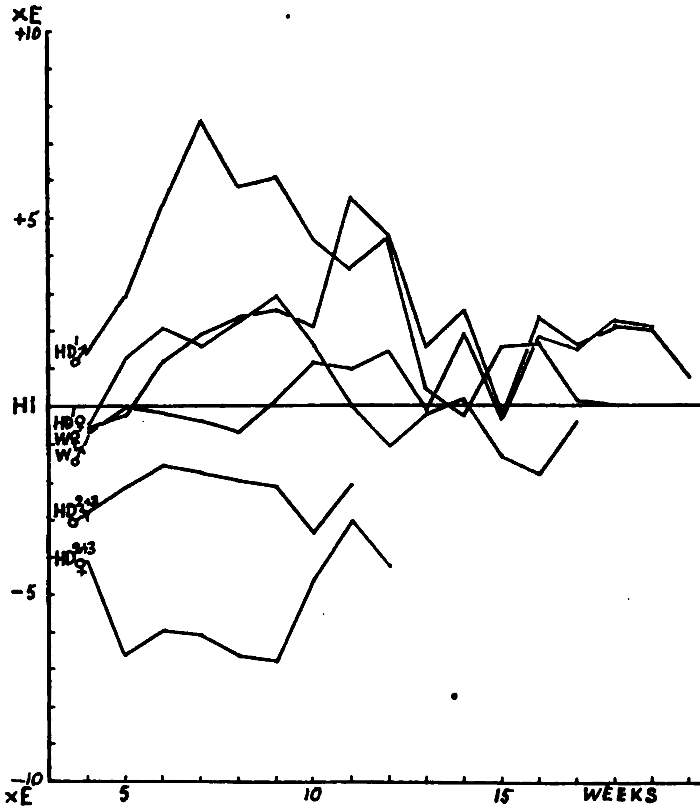


Fig. 4. Comparison of growth curves of "heat, stagnant air, descendants" and "wind" mice with "heat, immigrant" mice. Multiples of probable error of difference, *E*, between average weights. *HI* = heat, stagnant air, subdued light, immigrants; HD^1♂ = heat, stagnant air, subdued light, descendants, 1st generation, males; HD^1♀ = heat, stagnent air, subdued light, descendants, 1st generation, females; HD^{2+3}♂ = heat, stagnant air, subdued light, descendants, 2nd and 3rd generations, males; HD^{2+3}♀ = heat, stagnant air, subdued light, descendants, 2nd and 3rd generations, females; *W*♂ = heat, wind, males; *W*♀ = heat, wind, females.

It has repeatedly been found that, when the air is in motion, the overheating of the body which otherwise would occur in humid heat, is prevented. The effect of wind on growth has, as far as I know, not

Digitized by Google

been subjected to experimental investigation. My results in this respect point to a favorable influence. Although my material is rather limited and the "wind" curves in figure 4, therefore, do not deviate as much from the base line as would be desirable in order to definitely settle the problem, the fact that both the "male" and "female" curves

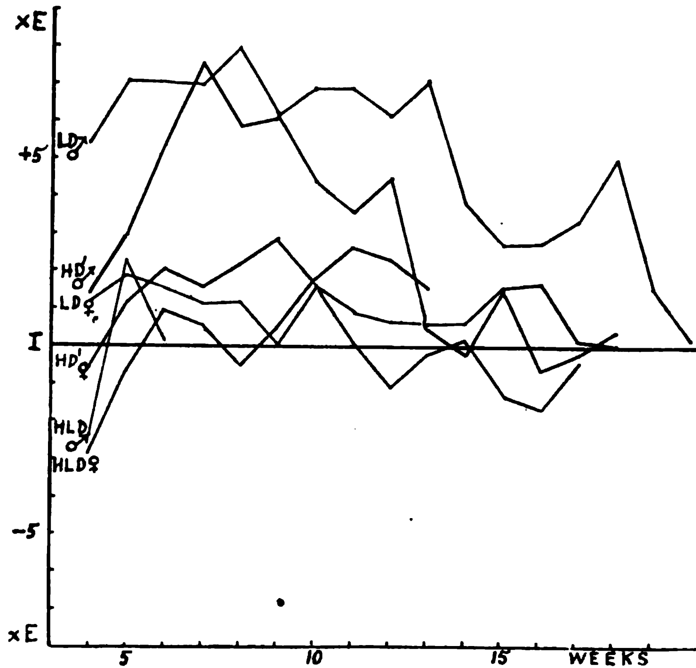


Fig. 5. Comparison of growth curves of "immigrant" and "descendant" mice in same environment. Multiples of probable error of difference, E, between average weights. I = Immigrant mice; LD ♂ = light, descendants, males; LD ♀ = light, descendants, females; HD^1♂ = heat, stagnant air, subdued light, descendants, 1st generation, males; HD^1♀ = heat, stagnant air, subdued light, descendants, 1st generation, females; HLD♂ = heat, stagnant air, light, descendants, males; HLD ♀ = heat, stagnant air, light, descendants, females.

are located on the plus side justifies us in assuming that circulation of air may play an important rôle in promoting the well-being of growing animals. In the interest of the physiological hygiene of the Tropics this phase of the subject deserves a thorough re-investigation.

It is a fact well known to students of the racial biology of white settlers in tropical countries that women are liable to suffer more

Digitized by Google

from the heat than men do. Although hygienic and social conditions may contribute to this difference in the power of adaptation between the sexes, the possibility is not excluded that the difference might have a biological significance. The fact that the female mice in my series reacted to the humid heat by a far greater retardation of growth and also exhibited less resistance in other respects to the new environment, deserves therefore to be mentioned. While the females seem to be more susceptible to agencies retarding growth, the male sex is apparently more easily affected by factors that neutralize the unfavorable environment.

Sufficient material seems to be at our disposal to disclose a marked difference in growth between mice that were transferred to a new environment when in a growing state, and mice that were born in the same environment from "immigrant" parents. This applies to climatic environments characterized by humid heat, radiant energy or both. A glance at figures 1, 2 and 5 will elicit this remarkable fact. In such of the three environments in which "immigrants" and "descendants" are compared the growth of the latter is more rapid. The degree of this difference between the generations seems to be proportional to the favorability of the environment.

Robertson (14) has published data that indicate that the retardation of growth that occurs in children as a result of an unfavorable environment is accompanied by a low variability of weight. This rule does not apply to our present study with exception possibly of the female mice that were born in the hot room and from birth exposed to strong light. In all the other hot room series the variability of weight is uniformly high. It is probable that this phenomenon may be the criterion of the existence of opposing climatic factors, some accelerators and others depressors of growth. When one of these groups of factors gains the upper hand the variability of weight seems to drop. The low variability of weight of mice that in the temperate room were exposed to light forms an illustration of the effect of the addition of a single factor acting as a growth stimulant.

My data concerning the fertility of the mice in the hot environment are limited. Since they give an idea of the relative fertility in several succeeding generations they deserve, however, to be reported. The total number of young born to twelve females that were transferred to the hot room in a pregnant state was 63. The average size of these litters is consequently 5.25. The average size of seven litters in the third generation of these mice was 5.57 and of eight litters in the fourth

Digitized by Google

generation 5.50. The average size of these 15 litters is 5.53. Robertson (15) gives 5.15 as the average of 241 litters of the same stock at ordinary room temperature. The average size of 50 litters I observed in the same climatic environment was six. These figures indicate that, notwithstanding the inbreeding, a slowly progressing increase of fertility had taken place. They further show that the heat mice had participated in this increase. Even considering the small material, we may say that at least no decrease of fertility had taken place. Steinach and Kammerer (3), from experiments on the effect of heat on the fertility of rats, report the average size of a litter at 20°C. as ten, at 25° as eleven and at 30° as eight. I am inclined to the view that the decrease of fertility in these experiments was due to the abnormally high normal fertility. My results indicate that when the fertility of the parent generation is of medium dimensions, a species may be capable of preserving this fertility uninfluenced by a hot environment.

SUMMARY

1. Exposure to artificial light at ordinary room temperature accelerates the growth of mice.
2. Confinement in a stagnant hot and humid atmosphere retards the growth of mice that were transferred to the new climatic environment immediately after separation from their mothers.
3. Succeeding generations of mice born in a hot and humid environment behave differently in their reaction to this environment. The first native generation may develop normally. The two next generations may elicit the greatest effect of the hot climate, at least as far as their growth is concerned. It is suggested that a racial adaptation may finally occur.
4. Exposure to artificial light in humid heat adds to the retarding effect on growth produced by the latter climatic factor.
5. Circulation of the hot and humid air neutralizes partly the unfavorability of the tropical environment for the growth of animals.
6. The growth of male mice is less retarded by an unfavorable environment and more accelerated by growth stimulating climatic fac torsthan the growth of female mice.
7. The growth of mice that are born in a new environment but the intrauterine development of which falls partly outside this environment grow faster than animals that are transferred to the same environment when in a growing state.

Digitized by Google

8. It is suggested that the higher variability of weight that occurs in an artificially produced tropical climate may depend upon the operation of opposing climatic factors of which one group may act as growth accelerators, the other as depressors of growth.

9. When the fertility of a mice colony is normal, it is not necessarily diminished by confinement of the mouse in humid heat for several generations.

BIBLIOGRAPHY

(1) PRZIBRAM: Verhandl. deutsch. Naturforscher und Ärtze, 81 Vers. Salzburg, ii, 1, 179.
(2) SUMNER: Journ. Exper. Zoöl., 1909, vii, 97; *Ibid.*, 1915, xviii, 325.
(3) STEINACH AND KAMMERER: Arch. Entwickl. Mech. Org., 1920, xlvi, 391.
(4) MITCHELL: Proc. Zoöl. Soc. London, 1911, 469; ref. ROBERTSON: Journ. Biol. Chem., 1916, xxiv, 356.
(5) ROBERTSON: Journ. Biol. Chem., 1916, xxiv, 363.
(6) ROBERTSON: Journ. Biol. Chem., 1919, xxxvii, 377.
(7) ROBERTSON: This Journal, 1915, xxxvii, 1.
(8) MALLING-HANSEN: Biol. Zentralbl., vii, 443.
SCHMIDT-MONARD: Journ. Kinderheilk., 1894, xl, 84.
(9) ALLEN: Science, n. s., 1905, xii.
BOETICHER: Zoöl. Jahrb., 1915, xl, 1.
(10) STEFANSSON: Journ. Amer. Med. Assoc., 1920, lxxv, 669.
(11) RUBNER: Energiegesetze, 1902.
(12) ALMEIDA: Journ. Physiol. et Path. gén., 1920, xviii, 713.
(13) ROBERTSON: Journ. Biol. Chem., 1916, xxiv, 374.
(14) ROBERTSON: This Journal, 1916, xli, 547.

Digitized by Google

STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

II. Relations of the Body Form and Especially the Surface Area to the Reactions Released by and the Resistance to a Tropical Climate

E. S. SUNDSTROEM

From the Laboratories of Biochemistry, University of California, Berkeley

Received for publication January 3, 1922

In the previous paper (1) I have suggested that the retardation of growth in a tropical climate may assist in promoting the cooling of the body by increasing the relative body surface. In this paper we will turn our attention to the possibility of an independent reaction of the peripheral parts of the body to the heat, which may be instrumental in changing the body surface:weight ratio in a direction favorable for the maintenance of the energy balance.

Sumner (2), in extensive investigations on mice, directed to establishing the inheritance of acquired characters, discovered an increase in length of tail, ears and feet in his hot room animals. This increase could be demonstrated also in mice that were born in the cold room of mothers which had lived in the hot environment. Sumner incidentally mentions the possibility that these changes might have some physiological significance but he makes no attempt to specify the advantage the animals may receive from them.

Rubner (3), in his work on the functions of the body surface in determining the basal metabolism, gives the surface:weight ratio of mice as 2.30. He has apparently left the area of the ears out of his calculations. It is evident, however, that Rubner, while assuming that the ears did not participate in the heat output in a cool environment, was aware of the fact that the ears—and also the tail—may serve as auxiliary cooling organs in a hot climate. I have found numerous occasions to confirm this hypothesis. The ears and the tail were in my hot room animals greatly hyperemic. Especially when the room temperature was allowed to rise to a height close to the body temperature, these external

Digitized by Google

parts were bright red. To the heat radiating organs I am also inclined to add the scrotum of male mice. An enlargment of the scrotum has repeatedly been reported in rats and mice that have been exposed to high external temperature. My heat mice formed no exception from this rule. In a rat colony that I have afterwards had opportunity to observe in actual Tropics every male had a hanging scrotum. It is obvious that such a sac of the integument, devoid of hair as it is, must offer for the animals an ideal cooling apparatus.

Considerations of this kind, naturally, do not find any human application. The possibility is, however, not excluded that even man may possess some means of increasing his surface: weight ratio. Since surface area as known is a function of height as well as of weight, an increase in the height: weight ratio will also result in an increase in the surface: weight ratio. The slender type of the aboriginals in the Tropics—with exception of Polynesians—may serve to illustrate this point. Observations in Java (4) on the height and weight of Dutch children have revealed that the height: weight ratio increases considerably faster in Java than in Europe. We may therefore infer that the surface: weight ratio for children of the same weight is higher in the former place.

My experiments to determine whether the surface: weight ratio undergoes any change in heat, when animals of the same weight are compared, were performed on two batches of mice, in which a number of litters were equally represented and of which one was kept in the temperate room until two months old and the other in the hot room up to the same age. Other environmental conditions were identical. Immediately after being killed, their weight and length were recorded, the latter measured without stretching from the tip of the nose to the tail end. The tail was measured from anus. One of the ears was cut out and trimmed along the protruding fold. It was then pasted on a piece of millimeter paper and the area computed. Tail and feet were cut from the carcass and their total skin area determined. The rest of the skin was finally carefully removed and spread out without unduly stretching on plate glass. A piece of millimeter paper was laid on top of the skin and with the empty side of the glass turned toward a window the contours of the skin were drawn as accurately as possible. The sum of the area of the skin plus the area of the ears—multiplied by 4—plus the area of tail and feet was taken as representing the total body surface.

Tables 1 and 2 contain data—for males and females separately—regarding body weight, total body surface, the constant in Meeh's surface

Digitized by Google

TABLE 1

Body weight, body surface, the constant in Meeh's surface formula, surface : weight coefficient, ear surface, ear surface in per cent of total surface, body length, length : weight ratio, tail length and tail length in per cent of total length in skinned male mice

NUMBER	BODY WEIGHT	BODY SURFACE	k IN $k\sqrt[3]{W^2}$	SURFACE : WEIGHT	EAR SURFACE	EAR : BODY SURFACE	BODY LENGTH	LENGTH : WEIGHT	TAIL LENGTH	TAIL : BODY LENGTH
					Controls					
	grams	*sq. cm.*		*sq. cm.*	*sq. cm.*	*per cent*	*cm.*	*cm.*	*cm.*	*per cent*
1	26.5	90.4	10.2	3.41	4.40	4.9	9.8	0.37	6.8	41.0
2	26.4	87.6	9.9	3.32	4.12	4.7	9.7	0.37	6.9	41.6
3	25.3	97.9	11.4	3.87	3.88	4.0	9.5	0.38	7.6	44.4
4	24.6	90.6	11.7	3.68	4.08	4.5	9.5	0.39	6.5	40.6
5	24.2	81.6	9.7	3.37	4.88	6.0	9.5	0.39	6.6	41.0
6	23.3	88.2	10.8	3.79	4.36	4.9	9.5	0.41	6.5	40.6
7	22.2	83.3	10.5	3.75	3.60	4.3	9.3	0.42	6.0	39.2
8	21.4	83.8	10.9	3.92	3.40	4.1	9.2	0.43	6.1	39.9
9	21.2	82.1	10.7	3.87	3.68	4.0	9.2	0.43	6.2	40.3
10	21.2	87.7	11.4	4.14	3.68	4.2	9.4	0.44	6.8	42.0
11	20.8	80.8	10.7	3.88	3.12	3.9	9.1	0.44	6.9	43.1
12	20.6	80.4	10.7	3.90	4.28	5.3	9.1	0.44	7.3	44.5
13	20.1	77.6	10.5	3.86	3.40	4.4	9.0	0.45	5.5	38.0
14	19.8	78.0	10.7	3.94	3.56	4.6	8.8	0.44	7.2	45.0
15	19.0	78.0	10.9	4.10	3.60	4.6	9.0	0.47	5.9	39.6
16	18.6	75.3	11.7	4.05	3.72	4.9	8.7	0.47	5.8	40.0
Average....	22.2	84.0	10.6	3.78	3.84	4.6	9.3	0.42	6.5	41.3
					Heat mice					
1	23.4	84.0	10.3	3.59	4.88	5.8	9.5	0.41	6.8	41.7
2	20.3	74.3	10.0	3.66	4.64	6.3	9.0	0.44	7.3	44.8
3	20.0	77.5	10.5	3.88	4.12	5.3	9.0	0.45	6.9	43.4
4	19.5	74.7	10.3	3.83	3.92	5.3	9.0	0.46	6.4	41.6
5	19.0	70.9	10.0	3.73	4.60	6.5	8.7	0.46	7.2	45.3
6	19.0	84.7	11.9	4.46	4.48	5.3	8.7	0.46	7.4	46.0
7	18.2	75.5	10.9	4.15	3.32	4.4	8.7	0.48	7.2	45.3
8	18.2	79.5	11.5	4.37	4.24	5.3	8.8	0.48	6.8	43.6
9	16.6	79.6	12.2	4.80	3.88	4.9	8.4	0.51	6.6	44.0
10	16.4	75.5	11.7	4.61	4.28	5.7	8.5	0.52	7.0	45.2
11	15.8	74.0	11.8	4.68	3.40	4.6	8.0	0.51	5.5	40.8
12	15.6	73.5	11.8	4.71	3.72	5.1	8.3	0.53	6.8	45.0
13	15.4	77.1	12.5	5.01	4.48	5.9	8.1	0.53	7.1	46.7
14	14.2	68.0	11.6	4.79	3.64	5.4	8.0	0.56	6.3	44.1
Average....	18.0	76.6	11.2	4.26	4.12	5.4	8.6	0.48	6.8	44.1

Digitized by Google

TABLE 2

Body weight, body surface, the constant in Meeh's surface formula, surface : weight coefficient, ear surface, ear surface in per cent of total surface, body length, length : weight ratio, tail length and tail length in per cent of total length in skinned female mice

NUMBER	BODY WEIGHT	BODY SURFACE	k IN $k\sqrt[3]{W^2}$	SURFACE WEIGHT	EAR SURFACE	EAR : BODY SURFACE	BODY LENGTH	LENGTH : WEIGHT	TAIL LENGTH	TAIL : BODY LENGTH
				Controls						
	grams	*sq. cm.*		*sq. cm.*	*sq. cm.*	*per cent*	*cm.*	*cm.*	*cm.*	*per cent*
1	21.3	71.4	9.3	3.35	3.56	5.0	9.0	0.42	6.8	43.0
2	20.2	74.1	10.0	3.67	3.84	5.2	9.0	0.45	6.0	40.0
3	19.3	71.9	10.0	3.73	4.36	6.1	8.8	0.46	7.0	44.3
4	18.9	68.3	9.6	3.61	3.64	5.3	9.0	0.48	6.2	40.8
5	18.6	71.8	10.2	3.86	3.76	5.2	8.5	0.46	6.5	43.3
6	18.6	74.0	10.5	3.98	2.96	4.0	8.7	0.47	6.4	42.4
7	17.7	72.8	10.2	4.11	3.76	5.2	8.8	0.50	6.3	41.7
8	16.6	69.3	10.6	4.17	3.80	5.5	8.8	0.53	6.0	40.6
9	15.9	62.4	9.9	3.92	3.72	6.0	8.3	0.52	5.7	40.7
Average....	18.6	70.7	10.1	3.80	3.72	5.3	8.8	0.47	6.3	41.7
				Heat mice						
1	23.7	84.2	10.2	3.55	4.72	5.6	9.1	0.38	8.0	46.8
2	17.7	76.9	11.3	4.34	4.92	6.4	8.5	0.48	7.1	45.5
3	17.4	67.5	10.0	3.88	3.92	5.8	8.7	0.50	6.7	43.5
4	17.0	70.9	10.7	4.17	3.92	5.5	8.5	0.50	6.9	44.8
5	17.0	71.0	10.7	4.18	4.24	6.0	8.4	0.49	6.5	43.6
6	16.9	70.3	10.7	4.16	4.60	6.5	8.6	0.51	6.0	41.1
7	16.6	70.8	10.9	4.27	4.28	6.0	9.0	0.54	6.5	42.0
8	16.5	69.3	10.7	4.20	3.96	5.7	8.0	0.48	6.4	44.5
9	15.0	65.5	10.8	4.37	4.08	6.2	8.0	0.53	6.2	43.7
10	15.0	66.8	11.0	4.45	4.16	6.2	8.1	0.54	6.8	45.6
11	14.9	66.4	11.0	4.46	4.04	6.1	8.3	0.56	6.2	42.8
12	14.3	68.0	11.5	4.76	4.20	6.2	8.0	0.56	7.0	46.7
13	14.1	63.2	10.8	4.48	3.84	6.1	8.4	0.60	5.8	40.8
Average....	16.6	70.1	10.8	4.21	4.24	6.0	8.4	0.51	6.6	44.7

formula, surface : weight coefficient, ear surface, ear surface in per cent of total surface, body length, length : weight ratio, tail length and the tail length in per cent of body length.

In conformity with the results of our growth studies we find a retardation of growth in the heat mice, which in this series in average amounts

Digitized by Google

to 4 grams for the males and to 2 grams for the females. The decrease of the average body surface is 9 per cent for the male and less than 1 per cent for the female sex. Calculated from Meeh's formula, body surface = a constant × cube root of squared body weight, a diminution of body surface, accompanying the decrease in weight, would have occurred, which in the male mice would have amounted to 15 and in the female mice to 8 per cent. This, supposing the same constant would be valid for both series of mice, which would be true if no change in body form had occurred in the heat mice. In Meeh's formula the constant is, as known, an expression of the body form and varies with different animals and to some extent also in the same species. We know that in Du Bois' formula for calculating the surface area of man the height has also been considered. For animals no such formula is available.

Rubner (3) has calculated the constant in Meeh's formula for the white mouse as 11.4. He found that after 4 days' starvation the factor rose to 12.3. We possess in this study all the necessary data for calculating the constant in both series of mice. For the male controls it varies between 9.7 and 11.7 and is in average 10.6. Corresponding figures for the heat male mice are 10.0, 12.5 and 11.2. The range for the control female mice is 9.3 to 10.6 with 10.1 as mean and for the heat female mice 10.0 to 11.5 with 10.8 as average. It is evident that in the heat mice the body form is instrumental in rendering available a larger cooling surface and that the male mice in both control and heat series are better qualified for cooling their bodies than are the female mice.

Regarding the relation of total superficial area to body weight it is seen that a larger skin area is available for each unit of body weight in the heat mice than in the temperate series. In the male control series the surface: weight coefficient varies between 3.32 and 4.14 and is in average 3.78. In the male hot room series the figures are 3.59, 5.01 and 4.26. In the female control series the mean is 3.80 with a range from 3.35 to 4.17, while the average in the hot room series for the same sex is 4.21 with a maximum of 4.76 and a minimum of 3.55. Considering averages we may say that an increase of cooling area had taken place in the "tropical" room that amounts in the male mouse to 12 and in the female mouse to 11 per cent. Since Moulton (5) has shown that body weight, surface area and amount of living protoplasm —as estimated from the nitrogen content—are functions of each other, we may assert that each gram of living substance in the hot environment has a larger cooling area at its disposal.

Digitized by Google

Since we know that a decrease in weight is accompanied by a relatively larger body surface it remains to consider whether the retardation of growth in the animals, which had been exposed to the hot environment, could, eventually be the sole cause of this phenomenon. That this is not true and that besides factors associated with the body form are responsible for the change, has already been made probable in our discussion of the constant in Meeh's formula. We may find further corroboration for this conception from a consideration of the size of the external parts of the body, viz., the size of the ears and the length of the tail. We find that the ear surface, in proportion to total body surface, has increased in the male mice from 4.6 to 5.4 per cent. In the female mice the relative increase of the ear surface is from 5.3 to 6.0 per cent. The length of the tail in relation to body length has increased from 41.3 to 44.1 per cent in male mice and from 41.7 to 44.7 per cent in female mice. Furthermore, the length : weight ratio has increased from 0.42 to 0.48 in males and from 0.48 to 0.51 in females. These results indicate that, irrespective of the change in body weight, morphological changes have taken place in peripheral parts of the body of the heat animals with a resulting enlargement of the body surface. In order to further confirm this conception I have in figure 1 plotted the surface : weight coefficients as ordinates, the abscissa giving the age in weeks.

As only a few individuals of lower body weight were available in the control series I have added half a dozen one week younger mice for comparison. With exception of a few cases—the probably rather large experimental error of the method must be considered—the control figures group themselves round almost a straight line. The curve for the heat mice again, while for higher ages at a low level, makes in direction of the younger mice a sharp bend upward indicating in these mice a high surface : weight coefficient. We find, consequently, that the retardation of growth in the heat mice has gone hand in hand with morphological changes of peripheral parts of the body which both ultimately result in supplying the organism with a larger cooling area per unit body weight.

It now remains to discuss the possibility whether an enhancement of the resistance toward heat actually occurs in those mice which have acquired the modified body surface. A convenient method of testing this point seems to consist in exposing animals that have had time to adapt themselves to moderate heat to excessive temperatures. My first experiment of this kind was an involuntary one. In a preliminary

Digitized by Google

hot room series, consisting of 23 mice, an accident allowed the temperature of the room to rise to 39° for one night two months after the commencement of the series. Next morning I found only four survivors in the lot, of which three were males. These were all individuals which had undergone a retarded growth. Figure 2 clearly demonstrates

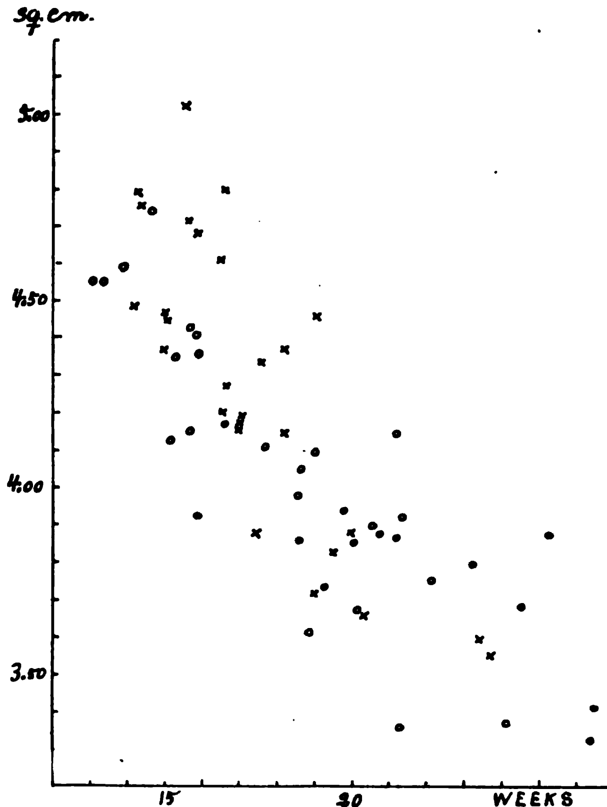


Fig. 1. Surface: weight coefficients and age. O = controls; X = heat mice.

the correlation of growth velocity and chance of survival in excessive heat.

In another experiment I tested the relative resistance to an excess of light—the heating effect of which was probably the primary factor—in two series of mice one of which had been bred in the hot room in subdued light, the other exposed to the light from a 100 watt "Mazda." The source of light in the experiment consisted of two 100 and two

Digitized by Google

60 watt "Mazda" globes, suspended about 40 cm. above the bottom of the cages. The air temperature was 33°, the relative humidity 60 per cent. A thermometer, the bulb of which was buried in the sawdust registered 38–39°. After 4 hours of exposure one third of the animals that had previously been protected from light survived the test. The proportion among the animals which had been accustomed to light was three in ten. One animal succumbed in the former batch

Fig. 2. Heat resistance experiment. Each of the 23 mice represented by a vertical growth line. *S* = survived; + = dead.

after only one hour of exposure. The other deaths occurred at intervals which were about the same in both cages. In the beginning of the experiment the animals drank freely of the water offered them and their movements were coördinated, later they ran around in a dazed condition. Apparently those mice that were most active succumbed quickest. The resistance did not seem in this case to be correlated by body weight. My impression that the central nervous system was primarily affected, that the deaths were due to real "sunstrokes" was supported

Digitized by Google

by post-mortem examinations. The meninges were highly edematous. The brain substance and the meninges showed numerous hemorrhages. Other typical signs of a "sunstroke" were also present.

A third heat resistance experiment in which I hoped to gain some information about the relative resistance of mice, which were adapted to different environments, to hot air, dry bulb = 30° of high humidity—near the saturation point—did not yield any conclusive result other than that mice succumb quickly in such a climatic environment. A short time after the commencement of the experiment the animals became covered with profuse perspiration and they all died at approximately the same time in all series. When the mice were weighed after death it was found that they had lost up to 10 per cent of their body weight in their futile efforts to affect body cooling by evaporation of water in the humid atmosphere. Sweating seemed in mice to be only an ultimate refuge in attaining the output of heat. Even at temperatures that were only slightly below body temperature animals which were adapted to heat were completely dry. Other individuals, however, which only for a shorter time had been exposed to the heat or which had been accustomed to the draught from a revolving fan were perspiring under these exceptional climatic conditions. If the temperature was not immediately lowered they soon succumbed.

SUMMARY

1. Retardation of growth may in a tropical climate assist in combating the overheating of the body. This effect is suggested as due to the enlargement of body surface area that accompanies the lower body weight.

2. Animals may be able by certain modifications of morphological characters, viz., enlargement of peripheral body parts, to further improve their cooling facilities.

3. A few experiments seem to indicate that, while a certain resistance to higher temperatures may be acquired by animals which have been adapted to external heat, this does not apply to environment in which light and humidity are the predominating climatic factors.

BIBLIOGRAPHY

(1) Sundstroem: This Journal, 1922, lx, 397.
(2) Sumner: Journ. exper. Zoöl., 1909, vii, 97; Ibid., 1915, xviii, 325.
(3) Rubner: Energiegesetze, 1902.
(4) Kiewiet de Junge: Mededeelingen Geneesk. Lab. Weltevreden, 1911, 2, Ser. A. 56.
(5) Moulton: Journ. Biol. Chem., 1916, xxiv, 299.

Digitized by Google

STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

III. Effect of the Tropical Climate on Growth and Pigmentation of Hair and the Dependence of these Integumental Functions on the Temperature Coefficient Law

E. S. SUNDSTROEM

From the Laboratories of Biochemistry, University of California, Berkeley

Received for publication January 3, 1922

In the previous paper (1) we have discussed some modifications which certain peripheral parts of mice undergo in a hot environment. The physiological usefulness of the enlargement of ears and scrotum and the elongation of the tail to the animals seems to be well established. The physiological factors that are at play in the development of these modifications are still obscure. The increase of blood flow through the integumental capillaries is probably one of these factors. Some evidence I have collected along other lines seems, however, to indicate that different histological components of the skin may be dependent in their proper functioning on different temperature coefficients. It is therefore possible that the extent of cellular activity that determines the size of above-mentioned peripheral parts may be a function of the external temperature. Although, naturally, the sequence of these events must largely escape direct observation, we will in the following find occasion to strengthen the hypothesis. Our attention in this paper will be chiefly directed to elucidating the rôle that temperature may play in hair growth and pigment production. By analogy the importance of the temperature coefficient law for the development of other integumental appendices will receive some support.

Rubner (2) has shown that the amount of hair is closely correlated to the effectiveness of the heat regulation of the body. We may therefore infer that in a tropical climate where demands are made upon an efficient cooling power of the skin the fur of animals should become thinner. This has actually been found to occur. Wild animal species extending over large areas from north to south have a thinner coat in the

Digitized by Google

southern part of their habitat (3). It is said that thoroughbred sheep in hot climates do not yield the same amount of wool as in the temperate parts of the same continent. Sumner (4) has experimentally verified this phenomenon. The total amount of hair he shaved from his cold room mice was consistently heavier than what he got from those mice which were adapted to a hot environment.

In connection with my measurements of the surface area of mice, reported in the previous paper, I found opportunity to compare the hair growth in two series of a genetically homogeneous mouse colony, one of which was kept at ordinary room temperature, the other transferred, when 3 weeks old, to a hot and humid environment. The mice were skinned at an age of 10 weeks and the total amount of hair removed with a safety razor. The average weight of hair of the control animals was 364 mgm. and of the heat mice 372 mgm. These figures indicate that the response of the mouse to external heat by thinning its fur may not be immediate. It must, however, be remembered that since the relative amount of moulting is impossible to determine, the experimental error in estimations of this sort must be rather large. It must further be borne in mind that in Sumner's experiments the control mice were exposed to considerably lower temperatures than was the case in my series.

In order to further test the promptitude of the effect of temperature changes on hair growth I have supplemented my mouse studies on this point with observations on the hair growth of a man for three periods during an eight months' stay in the Tropics, the first period commencing 2 months after arrival. The firmness of the hair of this subject made him especially suited for a study of this kind. The hair was at not too frequent intervals cut with machine to a standard length of 1 cm. During the first period—middle of December to middle of March—which in tropical Australia is characterized by a temperature round 30°C. and a relative humidity seldom below 80 per cent, the average daily hair growth was 161 mgm. During the next period—middle of March to middle of June—when the atmosphere was cooling off, the growth was 168 and during two winter months 176 mgm. The average temperature in winter was 20° with relatively low humidity. These results seem to indicate a dependence of hair growth on temperature, the maximum growth taking place inside the temperate range. The growth of nails was also observed in the same subject. In summer the average daily nail growth was 14.6 and in the cooler season 12.6 mgm. Could this stimulation of the growth of

Digitized by Google

finger and toe nails at high temperature be a parallel to the phenomena we referred to in the introduction of this paper?

I regret that I did not find time to repeat my weighings of the amount of hair in those generations of my mouse colony that were born in the hot environment. My impression was that the fur of the animals became progressively thinner, although even in later generations individuals were found that had a fluffy coat. The majority of mice in the fourth generation were almost hairless. Although we must not lose sight of the possibility that genetical factors may have coöperated in producing these skinny looking creatures—which nevertheless exhibited an almost normal velocity of growth—I am inclined to the view that environmental factors were chiefly responsible for their appearance. I find support for this view from the fact that about 2 weeks after the transfer of the same mice to a cool environment, they had acquired a fur that almost resembled a normal one. This tends to confirm our previous conclusion that the optimal temperature for hair growth is exceeded in a tropical climate.

In our inquiry into the temperature coefficients of different skin functions we now turn our attention to the effect of heat on pigmentation. An array of facts, anthropological and zoölogical, indicates that the intensity of integumental pigmentation may depend on climatic factors. The popular belief is that the intense solar radiation and primarily the actinic sun rays are the principal agents in producing the deeper coloration of animal species toward the equator. A number of experimental investigations has nevertheless been reported which point to an independent effect of increased temperature on pigmentation. Experiments on insects are in this respect somewhat conflicting, the best controlled investigations, however, indicating a darkening of color, in heat (5). Uhlenhuth (6), in his work on metamorphosis and pigmentation of batrachia, found that he could, by lowering or increasing the environmental temperature cause the specific coloring of Amblystoma tigrinum to disappear or become manifest. Bonhote (7) kept rodents of the species Meriones in humid heat for a few weeks, apparently protected from light, and observed a darkening of their coat color.

The coat color of mice has, as known, been a favorite field of research for geneticians. The conception of Cuénot (8) that at least two "factors" are necessary for color production, one representing the "chromogen" and the other the "zymogen," has considerably stimulated investigations along these lines. The conception was, however, a

Digitized by Google

qualitative one—presence or complete absence—and to conform with facts it was found necessary to introduce a number of color modifying factors. Cuénot explained albinism as a result of absence of either the chromogen or enzyme or both. Durham (9) concluded from experiments which have not been confirmed, that there may be present, in the skin of rabbits and rats, three specific enzymes for production of the black, yellow and chocolate color. She believed that these enzymes are lacking in albinos. Von Fürth's (10) suggestion that melanin is produced by the action of tyrosinase on tyrosin has given impetus to biochemical studies along these lines. Gortner (11) discovered that tyrosinase may be inhibited in its action by certain phenolic compounds and he thinks that such an inhibition is the underlying cause of the production of "dominant whites." Onslow (12) has confirmed this statement and found further that the pigmentation of an animal is a quantitative one, the same melanin giving all the shades from light yellow to black. The same investigator was unable to recover any tyrosinase from the macerated skins of recessive albinos but found that phenolases were present in appreciable amounts. It is noteworthy and gives rise to the suspicion that her extraction method, although it appears to be faultless, may not have been so, to hear that Onslow was also unable to obtain any tyrosinase from the skins of light yellow strains.

Riddle (13) has severely criticised the explanations geneticians give about the causes of albinism. He thinks that it is absurd to speak about gametes in which either the chromogen or enzyme is absent. I cite a few lines of his paper: "There can scarcely be any doubt that certain regions, owing to new structure, *new environment*, *new conditions* are able to oxidize different protein substances with variable ease and to a variable extent and even in a different way." And further: "The specific color of an animal is an index not of the processes in the germ from which this animal arose, of certain chromogens and specific zymogens, and the absence of wide series of others, but this specific color means that a process with a wide range of possibilities, because of a *particular physiological state and environmental conditions* has struck this particular equilibrium. *One and the same organism has within it all that is necessary to move the equilibrium up and down.*" The words in this citation that have important bearing on our present problem are italicized by me.

After this somewhat cursory review of a research field that still remains largely in an uncultivated state, I will proceed to report certain

Digitized by Google

facts with bearing on the pigmentation problem, which came to my attention during the course of my acclimatization studies. My material for these studies consisted of white mice that for a great number of generations had been found, by trained observers, to possess all the requirements of genuine recessive albinos. All my control series confirmed this classification. It therefore awakened my curiosity when I found that a relatively large number of individuals in certain well-defined experimental series were pigmented.

The modification in question was first discovered in a number of mice of the first generation which were born in the hot and humid environment. At first I paid no attention to the appearance of a creamy coat color in these mice, thinking that it was simply due to lack of cleanliness. This explanation proved to be incorrect. It was impossible to remove the color with any solvent. The possibility that particles of rust from the wire net were to blame was refuted by the iron test. The color commenced at the roots of the hairs. Doctor Hagedoorn, who is an expert on coat colors of mice, was kind enough to examine my mice and expressed as his opinion that they were truly pigmented.

The pigmentation made its first appearance when the mice were about 6 or 7 weeks old. In the hot room only mice belonging to the first "descendant" generation acquired pigment. It was later found that also a few mice which at ordinary room temperature had been exposed to strong electric light had become pigmented. All the pigmented mice were males. I was further able to ascertain that 75 per cent of them were individuals whose body weight was above normal. In the majority of cases the pigmentation was confined to the rump, ending in front along a rather sharp angular line. In a few instances a median, longitudinal creamy streak was observed on the belly. The region round the external sexual organs was deeply yellow. In other parts the pigmentation had a creamy tinge. It persisted mostly throughout the observed period of life of the animals. As might be expected, my attempts to test whether the pigmentation would breed true were negative.

For the microscopical examination of the hairs I employed Werneke's (14) method. The hairs were covered on a slide with a drop of glycerol and carefully heated in order to drive out air enclosed in them. I found that, primarily in the woolly hairs, golden yellow pigment granules were present in small numbers in the medullary part of the hair in places that corresponded to the position of pigment granules in yellow

Digitized by Google

mice. The grouping and dimensions of the individual granules were also identical. These groups of pigment granules extended as a rule along the whole length of the hair. In some hairs from the colored part of the coat these histological characteristics were missed, showing that these hairs were albinotic. The hairs from control mice were empty as far as genuine pigment granules are concerned. Occasionally, however, I could observe, near the base of a white hair, formations that answered the description Onslow has given about the "ground substance" in albino hairs (15).

The fact that the food of my mouse colony to a large extent consisted of yellow corn meal, which as known is rich in carotinoids, necessitated a few experiments directed to test the possibility that the yellow color was of such an origin. Its insolubility in ether and petrol ether seems to prove that neither carotinoids nor any lipochrome substance could be responsible for the yellow coloration. The albino rat has further been found to be devoid of carotinoids (16) and by analogy we may conclude that albino mice behave similarly in this respect.

A quantity of pigmented hairs was boiled with a 0.2 per cent sodium hydroxide solution for a while. After decanting the solution the process was repeated with a fresh amount of diluted lye. The two filtrates were poured together. The resulting liquid was bright yellow with a greenish tinge. After adding to one part hydrochloric acid and to the other saturated ammonium sulphate solution a greyish dark precipitate was thrown down in both cases. I am doubtful whether this could have been the "melanin." The amount was too small for chemical examination. Gortner (17) states that he has been able to obtain substances which answer the usual description of melanins, i.e., solubility in alkalies and insolubility in acids and neutral solvents from various keratin materials also from recessive albinos. He thinks that these substances do not belong to the true melanins. Gortner says further that he believes that most proteins contain a nucleus which, under proper conditions, may give rise to pigment.

I wasted much time in futile attempts to demonstrate the presence of tyrosinase in skins from my pigmented mice and also from a number of mice of varying age—a few days to several weeks—which were reared from birth in the "tropical" room. I adopted for this purpose Onslow's technic (12) in preparing the skin extracts. All my tyrosinase tests were negative. The presence of oxidases reacting with polyphenols could be demonstrated in the "heat" and "light" mice but the

Digitized by Google

intensity of the reaction in these series did not differ from that of the control mice.

Notwithstanding my negative results in demonstrating tyrosinase in the skins of the pigmented mice I am inclined to the view that enzymes —tyrosinase or others—which by reacting on protein substances in vivo may produce pigmentation were not entirely absent in either series of my material. I believe that the enzyme was present in all the animals in such a diluted state that it was destroyed during the process of extraction. If we assume that the optimum temperature for pigment production is relatively high, we may understand that the attenuated enzyme may be able, at higher temperatures, to react with the protein substrate while at lower temperatures it fails to do so. In this discussion we are primarily concerned with skin temperatures. As the height of skin temperature may be considered to be a joint product of internal and external factors we may comprehend the limitation of the colored mouse modification only to certain series of animals. It is possible that some correlation may exist between the pigmentation of the mice and factors that control the internal metabolism of the same. An incidental observation regarding the difference in reaction toward acetonitrile found in a few pigmented individuals which will be reported in the next paper seems to indicate that this may be the case. The fact that lecithin acts as an inhibitor on tyrosinase (18) may further have some bearing on the problem, since I have observed in some work in actual Tropics—as yet unpublished—that the lecithin of the blood undergoes a decided drop in hot climate.

I have recently found opportunity to observe in actual tropical climate a colony of white, pink-eyed rats, the ancestors of which several years ago were imported from Europe. These ancestors are said to have been pure white. At the present time the majority of the rats exhibit a fur of light yellow coloration, which is widely distributed over the coat. Among these pigmented rats the female sex is also represented but in a less degree. By microscopical examination the hairs from the pigmented rats show golden yellow granules identical to the ones I found in mice. Rats which since birth have been exposed to circulating air and whose cooling power was enhanced showed the pigmentation in a markedly less degree.

Reviewing our results on hair growth and pigmentation of mice we are justified in assuming that these closely allied processes are dependent on chemical reactions with different temperature coefficients.

Digitized by Google

SUMMARY

1. Mice which are suddenly transferred from a hot to a cool environment respond quickly to this change by a stimulated hair growth. No change of hair growth was found in mice that for 2 months had been exposed to humid heat. It is suggested that failure to observe the relative moulting of the mice may have obscured possible changes in direction of a thinning of the coat. Supplementary evidence is presented from observations on man to prove that the hair growth is more rapid in a cool environment.

2. The hair of recessive albino mice may, when exposed to humid heat or to the radiation of strong light acquire the power of producing pigment. This is in the beginning limited to individuals of a certain type, but as supplementary observations on rats indicate, may in subsequent generations extend to other individuals.

3. It is suggested that the prevalent theory that recessive albinos lack the pigment-producing enzymes is false and that failure to extract this enzyme may be due to its presence only in minute quantities.

4. It is further suggested that the chemical processes that control the growth and the pigmentation of hair may possess different temperature coefficients. While cool climate seems more congenial to hair growth the optimal temperature for pigment formation appears to fall within the range of tropical heat.

5. Finally, it is suggested that the small amounts of color-producing enzyme that are supposed to be present in the skin of albinos may become active only in a climatic environment in which the skin temperature of these animals approaches the optimal temperature for the reaction of the enzyme. The possible coöperation of metabolic factors is mentioned.

BIBLIOGRAPHY

(1) Sundstroem: This Journal, 1922, lx, 416.
(2) Rubner: Energiegesetze, 1902.
(3) Allen: Science, N. S., 1905, xii.
(4) Sumner: Journ. exper. Zoöl., 1909, vii, 97; Ibid., 1915, xviii, 325.
(5) Tower: Carnegie Inst. Publ., 1906, xlviii.
(6) Uhlenhuth: Journ. exper. Med., 1918–19, i, 527.
(7) Benhote: Vigour and heredity, 1915, 86.
(8) Cuénot: Arch. Zoöl. exper. et gén., 1903.
(9) Durham: Proc. Roy. Soc. London, 1904, lxxiv, 310.
(10) Von Fürth and Schneider: Hofmeisters Beitr., 1901, i, 229.
(11) Gortner: Journ. Biol. Chem., 1911–12, x, 416.

Digitized by Google

(12) Onslow: Proc. Roy. Soc. London, 1915–17, B, lxxxix, 36.
(13) Riddle: Biol. Bull., 1908–09, xvi, 316.
(14) Riddle and La Mer: This Journal, 1918, xlvii, 103.
(15) Werneke: Arch. Entwickl. Meck. Org., 1916, xlii, 72.
(16) Onslow: Knowledge, N. S., 1914, xi, 161.
(17) Palmer and Kennedy: Journ. Biol. Chem., 1921, xlvi, 568.
(18) Gortner: Journ. Biol. Chem., 1911–12, x, 115.
(19) Johnson: Univ. Cal. Publ. Zoöl., 1913, xi, 53.

Digitized by Google

STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

IV. Effect of Light and Heat on the Resistance of Mice to Acetonitrile

E. S. SUNDSTROEM

From the Laboratories of Biochemistry, University of California, Berkeley

Received for publication January 3, 1922

In the first one of this sequence of papers (1) evidence has been advanced to show that the growth of mice undergoes definite changes in a new climatic environment. Exposure to strong light at ordinary room temperature stimulated the physical development of mice, while on the other hand confinement in humid heat acted in the opposite direction. In the second paper (2) an attempt was made to attribute this retardation of growth solely to the requirement, in the interest of the energy balance of the animals, of an enlarged cooling surface. The possibility that growth might have only indirectly depended upon heat regulation by way of the effect this latter may have exerted on the activity of endocrine organs, primarily of the thyroids, was not considered. Since a retardation of general growth may result, as well known, from hyperthyroidism the possibility cannot *a priori* be excluded that the slow growth in the hot environment might have been caused by the presence in the blood of excessive amounts of the thyroid hormone. In this paper we will try, as far as the small material at our disposal will permit, to show that this was not the case. During the course of my investigations a considerable number of experiments was performed on the resistance of mice to acetonitrile, which has been found to be a convenient method of testing the thyroid activity. Most of these experiments have, however, been discarded, because the rule in testing the resistance to above-mentioned poison of injecting on the same day the same dilution of acetonitrile in mice of the main and the control series was not strictly adhered to in these experiments. It has been recently demonstrated (3) that the chemical undergoes changes in effectiveness with time even in unopened bottles. Only those experi-

Digitized by Google

ments in which these precautions were observed will, therefore, be reported.

In determining the thyroid activity of animals several methods are available. *a*, Comparing the relative increase in weight of the glands. Robertson (4) found that coincident with the principal cycles of growth of mice the thyroids increased in weight at a faster rate. This increase was more rapid in male mice which also exhibit a greater velocity of growth than females. It is doubtful, considering the small size of the thyroids in mice, whether it would have been possible to observe small deviations in the growth of these glands, such as possibly occurred in my environmental series. *b*, Estimating the iodine content of the thyroids. According to extensive observations published by Seidell and Fenger (5) the iodine content varies inversely with the size of the glands. Of great interest for our present inquiry is further their statement that both—size and iodine content—follow seasonal curves which for weight reaches a maximum in spring, synchronous with the minimum of the iodine curve. No figures are given on the body weight of these animals—sheep, cattle and hogs—but we may infer that the greatest increase of weight takes place in summer. *c*, Comparing the histological appearance of the thyroids. It has been found that the increase of iodine content of the glands coincides with typical changes of the vesicle epithelium and in the amount of colloid. The former becomes cuboidal and the vesicles themselves are distended by the colloidal content. Such changes, generally accepted as indicating a resting condition of the thyroid, have been observed by Mills (6) and by Stoland and Kinney (7) to result from exposure of animals to high temperatures. The latter investigators further observed that the toxicity of thyroid doses was enhanced by heat. *d*, Determining the resistance to acetonitrile. The three methods referred to above are undoubtedly useful in determining the condition of the thyroid gland itself. It is problematical, however, whether they are suitable for investigations which are primarily concerned with the relative amounts of thyroid hormone which are circulating in the blood. The acetonitrile method offers in this respect decided advantages. As known, it was originally introduced by Hunt as a measure of the extent of oxidative processes in the body (8). The rapid breakdown of the $CH_3 \cdot CN$ molecule with freeing of large amounts of the toxic hydrocyanic acid, resulting in diminished resistance, which was found in mice that had received repeated doses of ethyl alcohol, was explained by Hunt as commensurable with the acquired power of oxidizing the ethyl and methyl groups

Digitized by Google

at a faster rate than normal. The fact that the low oxidation rate, supposed to exist in inanition, was accompanied by an increase of resistance to acetonitrile seemed to support this view (9). In the hands of its originator, the acetonitrile reaction has later, as well known, been developed to a measure of the thyroid activity, in the first place of exogenous origin, but also of the normal physiological activity of the thyroid in the body (10). It seems to be fully established that the changes in oxidative power of the system Hunt discovered by his method are indirect effects of these physiological states on the thyroid. Since the chapters are not yet closed regarding the nature of the effect of the thyroid hormone we are not as yet in position to understand the mechanism of the acetonitrile reaction. It is believed by Hunt that some kind of neutralization process takes place between the two. A similar neutralization process presumably occurs normally between the thyroid secretion and various toxic compounds produced by the body metabolism, which act as depressors of oxidation. We know that the thyroid hormone itself stimulates oxidative processes. We possess, however, no information whatever regarding the interaction of these three classes of substances, the thyroid hormone, the toxic metabolites and acetonitrile. That essential differences exist between different species in this respect becomes evident when we recognize the fundamental dissimilarity between the reaction of mice on one side and of rats and guinea pigs on the other side toward acetonitrile after thyroid feeding. While the resistanceof mice is increased after such feeding, a diminution of the lethal dose occurs, as known, in the two other species of animals. Summing up these considerations, we may say that the acetonitrile reaction is an empirical test of thyroid activity and that great care must be exercised in applying results obtained in one species to other classes of animals.

For the understanding of our own results on the effect of climate on the resistance to acetonitrile it is fortunate that Hunt has collected material to determine the seasonal curve of this resistance (11). He found that the fatal doses of acetonitrile were for mice about twice as large in winter as in summer. Hunt doubted, however, whether the environment in which the mice were reared in winter—a room heated to 75°F.—could be classified as a cool environment. No figures are given for the summer temperature. References to the relative humidity and light conditions are also omitted. Knowing the dry atmosphere prevailing in heated rooms during a cold winter and on the other side the humid heat in the eastern states in summer, we may infer that

Digitized by Google

considerable differences must have existed between Hunt's summer and winter series so far as the heat output of the mice is concerned. The resistance of rats and guinea pigs was increased in summer, which may be easily understood, when the difference in behavior toward acetonitrile of these animals is considered. Hunt emphasizes the necessity of correlating the resistance with the rate of growth. He cites Südmersen and Glenny (12) who found that guinea pigs grew faster in summer than in winter and that it required a larger dose of diphtheria toxin to kill a guinea pig of same weight in summer than in winter. If Hunt had followed the actual growth of his animals he would possibly have found in mice a retarded summer growth, analogous to the retardation which I found in those of my mice which had been exposed to humid heat. It occurs to me that Hunt's winter series may correspond to my control series and that his summer experiments may in regard to climatic conditions be similar to my heat experiment.

For testing the effect of humid heat on the resistance of mice to acetonitrile I divided a number of litters into four batches, one male and one female group being kept at ordinary room temperature and the two remaining batches, males and females, being transferred to the hot room when 3 weeks old. The food was uniform in both series and consisted, as may be seen from the composition given in the first paper of this series, almost exclusively of food stuffs which according to Hunt's investigations (13) tend to increase the resistance of mice to acetonitrile. When the mice were 3 months old the resistance tests were performed on the same day with the same freshly made dilution of acetonitrile. The injections were given subcutaneously in the same place under the skin of the back. A few days later the same procedure was repeated with two batches of male mice, derived from the same litters, which had been reared at normal room temperature, one in subdued light and the other exposed to the light from two 60 watt "Mazda" lamps at close range. The age of these series of mice at the time of the test was three months. Tables 1 to 3 give particulars as to dosage of acetonitrile, body weight of the mice and result of injection.

The lethal dose in all the series is very small and corresponds to the minimal fatal doses observed by other investigators in this field. How much this low resistance of my mice might depend upon specific qualities of them and how much, as far as the controls are concerned, upon climatic effects is impossible to tell. The fact that the fatal dose reported by European observers (14) is consistently higher than in America may suggest that the latitude may play a rôle in determining the

Digitized by Google

resistance of mice to acetonitrile. It is possible, however, that simply the degree of toxicity of the acetonitrile preparations used by different investigators is responsible for the variations in fatal dose.

The slight difference we notice in the two first tables toward an increase in resistance in favor on one side of the female mice as compared

TABLE 1

Resistance of male heat mice to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT		RESULT	
	Heat mice	Controls	Heat mice	Controls
mgm.	*grams*	*grams*		
0.10	19.5	22.0	Survived	Survived
0.15	18.5	22.3	Survived	Survived
0.20	24.8	22.9	Dead	Survived
0.22		25.9		Dead
0.25	20.8	25.0	Dead	Dead
0.30	18.5		Dead	
0.35	19.0		Dead	
0.40	22.3		Dead	
0.45	21.7		Dead	

TABLE 2

Resistance of female heat mice to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT		RESULT	
	Heat mice	Controls	Heat mice	Controls
mgm.	*grams*	*grams*		
0.10		21.5		Survived
0.12		22.7		Survived
0.14	15.2	20.3	Survived	Survived
0.16	18.9	17.8	Survived	Survived
0.18	22.8	23.5	Survived	Survived
0.20	16.1	18.7	Survived	Survived
0.22	19.3	19.3	Survived	Survived
0.24	17.4	22.0	Dead	Survived
0.26		19.3		Dead

with the male ones and on the other side of heat animals above controls, is probably too insignificant to deserve any attention. We are, however, justified in concluding that no increase of the resistance to acetonitrile occurs in mice which for the greater part of their lives have lived in an artificially produced tropical climate.

Digitized by Google

The resistance in the "light" series was decreased by one-third as compared with the corresponding control series. Notwithstanding the small absolute difference, I am led to believe that this result may be accepted as indicating a diminution of resistance in strong light. On account of the small number of animals this conclusion, naturally, requires confirmation. In this connection I take the opportunity of reporting an experiment performed about two months afterwards on seven male mice which all, since they were three weeks old, had been exposed to electric light. Two of these mice belonged to the "pigmented albinos" which have been discussed in a previous paper (15). The animals were about 5 months old at the time of the experiment. The

TABLE 3

Resistance of male "light" mice to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT		RESULTS	
	Heat mice	Controls	Heat mice	Controls
mgm.	*grams*	*grams*		
0.09	23.2		Survived	
0.10	22.3	24.2	Dead	Survived
0.11	23.2	20.7	Survived	Survived
0.12	22.0	18.5	Dead	Survived
0.13	20.6	20.9	Dead	Survived
0.14	23.4	21.3	Dead	Survived
0.15	24.2	20.6	Dead	Dead
0.16	24.8	20.6	Dead	Dead
0.17		21.3		Survived
0.18		24.8		Dead
0.19		22.6		Survived

injections of acetonitrile were given a few days after the discontinuance of the light treatment. It is probable that the acetonitrile had at that time lost a part of its strength. This would explain the great difference in fatal doses between these experiments and the previous "light" experiments. One of the pigmented mice was given 0.20 and the other 0.25 mgm. per gram body weight. One of these mice, ear markings omitted in the protocol, succumbed quickly. The other lay in a stupefied condition for 12 hours but finally recovered. The unpigmented mice were not at all affected by doses twice as large. This experiment seems to suggest that metabolic factors, the exact nature of which it would be impossible to conjecture, were coöperating in producing the pigmented modification in my mouse colony.

Digitized by Google

It would have been highly desirable to obtain exact figures with regard to the effect of heat on the resistance to acetonitrile in later generations of mice which were born in the hot environment. Several protocols of experiments of this kind were discarded for reasons that have been referred to above. I am, however, still in possession of a protocol of an experiment on eight female mice from the third generation which were born in the hot room. These tests were performed about the same time as the tests on the pigmented "light" mice, which have led us to conclude that the stock solution of acetonitrile had lost part of its effectiveness. The age of these "descendants" of the third generation was 3 months, the same as that of animals which were subjected to the test after having lived in the hot environment from 3 weeks of age. Their body weights are very much lower. In table 4

TABLE 4

Resistance of female heat mice in the third generation to acetonitrile

DOSIS PER GRAM BODY WEIGHT	BODY WEIGHT	RESULT
mgm.	*grams*	
0.10	17.0	Dead
0.15	14.9	Survived
0.15	16.0	Survived
0.20	15.0	Survived
0.25	12.4	Dead
0.30	16.4	Survived
0.33	15.0	Dead
0.35	18.2	Dead

the data are collected for this incomplete experiment. The variability of the lethal dose is conspicuously high. The data, even without any correction for the change in toxicity of the chemical, do not lend any support to the supposition of a diminution of the resistance to acetonitrile in a hot climate. It rather appears as if, actually, the effect were an opposite one, which would tend to accentuate the slight indications in a similar direction which we received in our better controlled heat experiment of this sort.

Translating our results into terms of thyroid activity according to our accepted principles we may say that confinement of mice even for several generations does not increase this activity but rather acts in an opposite direction. Exposure to strong light seems to cause a slight diminution of the thyroid hormone in the blood. These results seem

Digitized by Google

further to be in full accord on one side with the seasonal curve of thyroid activity that Hunt has reported, and on the other side, with regard to the heat experiments, with the findings of investigators who have furnished histological evidence of a resting condition of the thyroid in a hot environment. Whether these changes form a link in the adaptation mechanism of the body to a tropical environment is impossible at present to say. It is possible that the demand for active thyroid hormone is diminished in the Tropics, where the "inside fires are banked." Observations that an increase in external temperature enhances the toxicity of thyroid medication seems to indicate that the body might in a hot climate instinctively lessen the flow of thyroid secretion into the blood. Although it must once more be emphasized that experimental results obtained on animal species cannot directly be applied to human medicine, we are nevertheless compelled to be sceptical about certain "clinical" reports of tropical practitioners who announce wonderful benefits from the use of thyroid preparations on various conditions of debility.

The question which was the immediate origin of this ingress into the field of internal secretion, namely, whether the retardation of growth of mice, which are adapted to a humid and hot environment, may be attributable to a stimulation of thyroid secretion must be answered in the negative.

SUMMARY

1. The resistance of mice to acetonitrile is not augmented in humid heat; the small material at hand indicates on the contrary a slight diminution of this resistance. It is, therefore, suggested, in the light of accepted principles regarding the correlation of acetonitrile resistance and thyroid activity, that the tropical climate—at least for mice—may lessen the demands for thyroid hormone in the body.

2. The retardation of growth of mice which has been observed to occur in humid heat is not attributable to a stimulation of the thyroid activity.

3. The resistance of mice exposed to strong light at ordinary room temperature is slightly diminished.

BIBLIOGRAPHY

(1) Sundstroem: This Journal, 1922, lx, 397.
(2) Sundstroem: This Journal, 1922, lx, 416.
(3) Wuth: Biochem. Zeitschr., 1921, cxvi, 237.

Digitized by Google

(4) Robertson: Journ. Biol. Chem., 1916, xxiv, 377.
(5) Seidell and Fenger: Hyg. Lab. U. S. Pub. Health Mar. Hosp., 1914, Bull. 96.
(6) Mills: This Journal, 1918, xlvi, 329.
(7) Stoland and Kinney: This Journal, 1919, xlix, 135.
(8) Hunt: Hyg. Lab. U. S. Pub. Health Mar. Hosp. Ser., 1907, Bull. 33.
(9) Hunt: Hyg. Lab. U. S. Pub. Health Mar. Hosp. Ser., 1910, Bull. 69.
(10) Hunt and Seidell: Hyg. Lab. U. S. Pub. Health Mar. Hosp. Ser., 1909, Bull. 47.
(11) Hunt: Hyg. Lab. U. S. Pub. Health Mar. Hosp. Ser., 1909, Bull. 69, 74.
(12) Südmersen and Glenny: Journ. Hyg., 1909, ix, 399.
(13) Hunt: Hyg. Lab. U. S. Pub. Health Mar. Hosp. Ser., 1910, Bull. 69, 51.
(14) Trendelenburg: Biochem. Zeitschr., 1910, xxix, 396.
Port: Biochem. Zeitschr., 1913, li, 224.
Wuth: Biochem. Zeitschr., 1921, cxvi, 237.
(15) Sundstroem: This Journal, 1922, lx, 425.

Digitized by Google

STUDIES ON THE ADAPTATION OF ALBINO MICE TO AN ARTIFICIALLY PRODUCED TROPICAL CLIMATE

V. Effect of Humid Heat on the Blood Morphology of Mice

E. S. SUNDSTROEM

From the Laboratories of Biochemistry, University of California, Berkeley

Received for publication January 3, 1922

Probably the first contribution of scientific value to tropical physiology was made in the realm of blood morphology. Among medical as well as laymen the conception had been prevalent that the tropical climate as such may affect the blood conditions and cause a "tropical anemia." A number of short, more or less well-controlled investigations was therefore made on the blood picture of individuals who for a time had resided in the Tropics. They all seemed to refute the existence of an anemia of purely climatic etiology. These results have at a later date been confirmed by more extensive and systematic investigations (1). As to the red blood corpuscles, some observers have gone so far as to postulate that, in analogy with experiences in high altitudes, the number of erythrocytes may as a rule be increased in healthy inhabitants of the Tropics (2). Still less consensus of opinion exists with regard to the white blood corpuscles. While most reports (3) indicate that their number generally lies in the lower part of the range for temperate climates, other investigators (4) are inclined to the view that a slight hyperleucocytosis is produced by heat. A few observations (5) which point to the existence of lymphocytosis and a shift to the left of the Arneth index, have been criticised as paying not sufficient attention to the possible effects of parasitological ailments, and on the other hand not considering various climatic conditions and seasonal variations. It is surprising that, so far as I am aware, no attempts have been made to approach these problems by experiments on animals, living for longer periods of time in an artificially produced tropical environment. Of considerable interest in this connection is, however, a research series published by Murphy and Sturm (6). These investigators found that after exposure for 5 minutes to dry heat—55 to 65°C.—the blood picture of rats, mice and guinea pigs

Digitized by Google

underwent decided alterations. The white blood count dropped and this change affected equally the mono- and poly-nuclear cells. During the days succeeding the transfer back to a cool environment the curves commenced to rise and reached finally levels far above the original counts. The lymphocytes were primarily affected by this compensatory change. It was further found by Nakahara (7) that after the heat treatment numerous degenerated cells were present in the spleen and in the lymphatic glands. An enhanced cell proliferation was discernible during the after-period.

It occurred to me that my healthy and well-controlled mice series might offer an ideal material for studies on the effect of humid heat on the blood morphology. Insofar as time and available animals permitted I therefore gave my attention to this line of research. As the customary method of drawing the blood from the tail vein of mice seemed to me to be open to criticism, I decided to let my blood samples represent as large part of the total blood volume as possible. I therefore killed the animals by cutting their throats and collected the freely flowing blood on a watch glass. After quick mixing of the blood the blood pipettes were filled within a few seconds after the operation. A few times control countings were made by filling two pipettes from the same blood sample with satisfactory agreement between the results. While thus the method, with regard to blood counts, was fully satisfactory and obviously superior to the employment of peripheral blood for the purpose, I regret to say that the blood smears—four or five from each mouse—did not fulfil my expectations. Differential counts obtained from different slides for the same animal did not agree. Most probably the time consumed in preparing the slides was sufficient for the blood cells to commence to settle down. Stirring did not completely restore the original distribution of different classes of white cells. Under those circumstances it would have been waste of time to continue differential counts, the results of which would have been uncertain. I gained, however, the impression that the mononuclears —especially the large ones—were relatively numerous in both series, heat and control. In the latter they seemed to exceed the figures published by other observers (8). I abstained also from a classification of the polynuclears, since this apparently offers considerable difficulties in mice. I received from a few comparative observations the impression that more rod nucleated polymorphonuclears in proportion to polymorphonuclears with a vacuolized or pycnotic nucleus were found in the mice from the hot environment than in the controls.

Digitized by Google

Red blood corpuscles. In ten control mice the average red count was 10.14 million with a standard deviation of 0.75 million. Since the red counts of males and females agreed in all respects I did not attempt to treat them separately. The sexes were furthermore equally represented in all series. The number of erythrocytes of the controls agrees well with standard figures reported by Klieneberger and Carl (8), average 9.73 million, and by Lange (9), males 9.48 and females 9.18 million. In 16 mice which had been exposed to heat since they were 3 weeks old the red count was 11.16 million with a standard deviation of 1.16 million. An increase of 10 per cent had consequently taken place in the average red count of the heat mice. The chance that this increase was accidental is one in 142. If the eight red counts that were performed on various other series of "immigrant" heat mice are added the average of the 24 variates remains the same, 11.13 millions. The average of eight red counts on mice which were born in the hot environment was 12.10 million which seems to indicate that a progressive change in a positive direction of the number of erythrocytes took place in the "descendant" generations. The greater part of this rise of the erythrocyte curve is doubtless due to inspissation of blood but, considering the synchronous progressive drop in the total white count, which will be referred to below, the possibility cannot be excluded that a real increase of small dimensions might have occurred in the number of red corpuscles in those mice which were adapted to a hot and humid environment.

White blood corpuscles. The table below gives a comprehensive idea of the changes of the total white count in mice which were reared in the "tropical" environment. The average count for the control animals closely resembles the standard figure given by Klieneberger and Carl (8) 7400. The number of white blood cells of "immigrants" in the hot room is lower but the probable error of the difference is too large to allow any definite conclusion to be drawn. Both series of the first generation born in the hot environment—one kept in subdued light and the other exposed to the light from a 100 watt "Mazda" at close range—agree closely with regard to their avarage white count. The chance that the difference between the controls and these "descendants" would be accidental is one in 100. The white count of the fourth generation is still lower.

A correction for the inspissation of the blood in the mice from the "tropical" room would only serve to accentuate the difference in white count between the controls and the heat mice. We seem to be justified,

Digitized by Google

therefore, in assuming that the heat tends to lower the number of white blood corpuscles, which is in agreement with the majority of observations on human blood in the Tropics. We are led by these results to believe that the organs concerned in the formation of white blood cells are sensitive to tropical heat. In the experiments from the Rockefeller Institute which we have referred to above, the extreme heat stagnation lasted only for a very short time. Under the climatic conditions that prevail in the Tropics and which we have imitated in these studies the moderate heat stagnation is continuous. The results seem to be the same in both cases with regard to the effect of the heat on the cellular activities that control the supply of white blood cells. It is regrettable that I omitted to consider the effect on the white count a transfer of the mice to a cool environment would have caused. It is

TABLE 1

Average white count with standard deviation in control and four series of heat mice

SERIES	NUMBER OF VARIATES	MEAN	STANDARD DEVIATION
Control mice	38	7300	2170
Heat mice, "immigrants"	13	6880	3170
Heat mice, "descendants" 1. generation subdued light	25	5730	2010
Heat mice, "descendants" 1. generation strong light	25	5550	2050
Heat mice, "descendants" 4. generation	25	4950	1730

possible that those investigations of human blood in the Tropics which have showed a hyperleucocytosis—supposing pathological factors were absent—have been made primarily during cooler respites and that the increase of white cells they indicate is analogous to the hyperleucocytosis which Murphy and collaborators observed as an after reaction to the hot air treatment of their animals.

SUMMARY

1. Progressive changes in direction of an increase of the number of erythrocytes were observed in mice which for the greatest part of their life or since birth had been confined in humid heat. It is suggested that this change is primarily due to inspissation of blood.

2. Progressive diminutions of the white count occurred in a number of generations of mice which were reared in humid heat. It is suggested

Digitized by Google

that this change was produced by high sensitivity to an increase of temperature exhibited by organs concerned in new formation of white blood cells.

BIBLIOGRAPHY

(1) CHAMBERLAIN: Philip. Journ. Science, 1911, vi, 483.
BREINL AND PRIESTLEY: Ann. Trop. Med., 1914, viii, 591.
(2) MARÉSTANG: The Practitioner, 1891; ref. SCHILLING-TORGAU, see below.
(3) SCHILLING-TORGAU: Menses Handbuch d. Tropenkrankheiten, 1914, ii, 58.
(4) BREINL AND PRIESTLEY: Ann. Trop. Med., 1914, viii, 565.
(5) CHAMBERLAIN AND VEDDER: Philip. Journ. Science, 1911, vi, 405.
(6) MURPHY AND STURM: Journ. exper. Med., 1918, xxix, 1.
(7) NAKAHARA: Journ. exper. Med., 1918, xxix, 17.
(8) KLIENEBERGER AND CARL: Blutmorphologie d. Lab. Tiere, 1912, 5.
(9) LANGE: Zoöl. Jahrb., 1919, xxxvi, 657.

Digitized by Google

STUDIES IN PLACENTAL PERMEABILITY

II. Localization of Certain Physiological Activities in the Chorionic Ectoderm in the Cat

R. S. CUNNINGHAM

From the Department of Anatomy, Johns Hopkins University

Received for publication January 4, 1922

The first communication of this series concerned a group of experiments the results of which were of interest in two of the many phases involved in the complex question of nutrition of the fetus. In these experiments it was found that both iron ammonium citrate and sodium ferrocyanide, when given in a solution containing equal amounts of each salt, passed through the easily permeable maternal endothelium in ten minutes. Both of these salts penetrated the syncytial layer of the chorionic ectoderm in about two hours, and finally the ferrocyanide reached the fetal circulation in four or five hours while the citrate had not done so at the end of ten.

When the placentae and membranes from these animals were fixed in an acid medium Prussian blue granules were precipitated in characteristic regions of the chorionic ectodermal layer. Each giant cell was surrounded by a ring of blue granules and both the portion of the syncytium bordering the maternal endothelium and some of the fine strands of cytoplasm which appear between the large vacuoles, seen in the fixed material, also contained the Prussian blue. The distribution of the granules of Prussian blue indicated the distance to which the citrate had penetrated. The area in which these granules were found seemed therefore to have special significance, and this finding suggested that perhaps this was an area in which some particular physiological activity took place, perhaps the breaking down and resynthesizing of substances finally intended for fetal consumption. On the other hand the fact that these two salts, which diffuse at approximately the same rates through inert membranes, should show such different properties when brought into contact with the placental membrane, suggested that there must be some factors involved in placental inter-

Digitized by Google

change other than the simple diffusion of molecules in the physical sense. Several suggestions were made as to the possible nature of these additional factors, the most plausible being that the chorionic ectoderm was capable of breaking down the citrate in such a way that the ferric iron no longer reacted as such.

Two very interesting papers, appearing too late to be referred to in the first number of this series, are important in relation to the two principal conclusions which have been outlined above. Wislocki (37) has studied the distribution of trypan blue in the placenta of the cat, after administration to the mother in repeated doses. It may be seen by looking at his figures 14 and 15, plate 4, that the distribution of the droplets of trypan blue in the syncytium of the fetal ectoderm is quite similar to that which I have described for the Prussian blue. This fact is interesting because it demonstrates that this particular area of the fetal chorionic syncytium is capable of reacting to at least two types of substances, one of which is an inert colloid and the other a diffusible salt. From these observations it may be safely suggested that much of the normal physiological activity of the placenta is localized in this area.

The other paper which is of such particular interest in this connection is one by Edelstein and Ylppö (7). These authors made comparative studies of the blood of the mother and the newborn child. They determined the amount of sodium and potassium in both bloods; and found that in all cases the sodium content was greater in the fetal blood than in the maternal. In nine cases they also found that the amount of potassium was larger in the fetal blood than in that of the mother, but there were three interesting exceptions; in two of these the maternal content was much higher, the fetal remaining exactly the same as in the other nine cases. The abnormally high percentage for the maternal blood in these two cases the authors attribute to nutritional factors. In the third case where the usual ratio was not found, the content of the maternal and fetal bloods in potassium was the same. In this instance the mother and child were both syphilitic and the authors suggest that the normal regulating mechanism of the placenta may have been impaired so that the salts could pass more easily, perhaps even as in the case of an inert membrane. They consider that their results give evidence that the passage of salts from mother to fetus is not controlled entirely by the laws of osmosis and diffusion, and suggest that the mechanism of placental transfer is regulated by certain vital cellular activities similar to those obtaining in intestinal and kidney cells.

Digitized by Google

The interchange of salts and the localization of activities in the placenta are two of the most essential points to be considered in studying the phases of the activities in which that membrane is called upon to mediate. Many possible explanations of these phenomena have been suggested, yet none of these can be entirely correlated with the great mass of evidence which has accumulated on the subject.

This number of the series is intended to examine the reaction of the cat's placenta after more extended exposures to sodium ferrocyanide and iron ammonium citrate than those reported in the first paper, to control the interactions of the two salts by studying them separately, and to further control the use of anesthetics by several new methods. With these facts established as far as possible with the limited material at hand some attempt is made here to determine the exact bearing of these experiments upon the question of placental interchange and to establish their proper relationship to the other work which has been done.

Materials and methods. The animals used were pregnant cats, in the latter half of gestation, the youngest fetus measuring 79 mm. and the oldest 110 mm. The methods employed were in general similar to those recorded in the earlier communication (5), and were briefly as follows: the animals received balanced solutions of sodium ferrocyanide and iron ammonium citrate, the usual strength was 1½ per cent of each salt. The solution was injected into the vein of the fore-leg, the temperature and rate of flow being kept constant. At the termination of the experiment the animal was killed, the uterus opened and the fluid carefully withdrawn from the amniotic sac with a syringe and needle. The fluid was then tested for the experimental salts. The fetuses were removed and their abdominal cavities opened. If the bladder was distended, it was handled as in the case of the amniotic sac. The placenta and membranes were fixed in Bouin's fluid, the acetic acid in the mixture precipitating the Prussian blue. In the present series of experiments some placental tissue was also fixed in neutral formalin and 95 per cent alcohol. In the analysis of the amniotic liquid and fetal urine, solutions of ferric chloride and ferric sulphate were used to test for the ferrocyanide, and ferrocyanide was used to determine the ferric iron. The longest experiment in the first series was 10 hours. In this series the duration of the experiments was extended to 14, 18 and 24 hours. No animals were kept exposed to either or both of the two experimental salts longer than 24 hours. These experiments of 14, 18 and 24 hours were on animals which had been nephrectomized under

Digitized by Google

ether anesthesia, the salts injected intravenously and the animals allowed to recover. They remained entirely normal in appearance up to about 20 hours when they became slightly drowsy.

A few other experiments using each of the two salts alone were carried out to determine whether the sodium ferrocyanide had any part in preventing the passage of the iron ammonium citrate through the placenta. Finally several experiments of 4 to 10 hours duration both on animals which had been nephrectomized and on others with functioning kidneys, were done under urethane. These experiments were carried out as controls for those done under ether, since urethane has been found to be practically without effect on respiration, circulation, etc.

Experimental results. On histological examination, the placentae from those experiments in which a combined injection of sodium ferrocyanide and iron ammonium citrate was given, presented the same general features as those described for the shorter experiments. In all of them in which the fetuses were alive when the animal was sacrificed the Prussian blue could be seen surrounding the giant cells in a definite blue ring, a few granules of blue could occasionally be seen within these cells, but these were quite rare.

The maternal endothelial cells contained a few blue granules within their cytoplasm, these were however less in number than those found in experiments of 2 to 4 hours duration. The striking finding was again the appearance of the syncytium of the chorionic ectoderm: here the Prussian blue was precipitated in clumps and fine lines within the cytoplasm adjacent to the maternal endothelium and extended between the nuclei, sometimes as far as the end of the nucleus nearer the fetal vessels. The border of the chorionic ectoderm adjacent to the fetal vessels always remained free of the Prussian blue.

In most of the experiments having a duration of more than 8 hours there was evidence of some precipitation of Prussian blue in the living placenta, but this was never extensive. In these cases some of the tissue was always fixed in neutral formalin or alcohol and care was taken that no acid reagent came in contact with the sections during staining. In this way the amount and distribution of this precipitation has been determined. Sections from these placentae showed the usual distribution of Prussian blue but the amount was very greatly reduced, usually there were a few fine granules between the maternal endothelium and the chorionic ectoderm and others distributed in the border of the syncytial layer of the chorionic ectoderm adjacent to the maternal circulation. These observations are interesting because they indicate the

Digitized by Google

involvement of some factor that brings about a partial precipitation of the Prussian blue during the life of the cells. That this is true, (i.e., that the chorionic ectodermal cells are still living when the precipitation takes place) is evidenced by the totally unstained state of the nucleus. In all histological preparations from the experiments of this series and those reported before, a few nuclei can be found that have stained a diffuse blue; these unquestionably indicate cellular death, but there has been no evidence of any increase in the number of stained nuclei in the experiments of longer duration or in those in which the maximum degree of intra-vitam precipitation has been found. This observation indicates that the degeneration of the protoplasm and the consequent production of acid can not be admitted as a likely explanation of the partial precipitation of the blue granules.

Turning now to the experiments of longer duration in which the citrate was used alone, only three animals were available for this purpose. In two of them double the usual dose of the iron ammonium citrate was given and the animals sacrificed at 12 and 18 hours respectively. In neither case was there any evidence of ferric iron in the urine of the fetuses or in the amniotic fluid. In the third it was thought interesting to try a very large dose with the hope of overloading the placental mechanism and then finding the passage of ferric iron to demonstrate that the membrane as such was permeable to the simple salt. In this animal 4½ grams were injected intravenously into the circulation of the mother in a 2 per cent solution. The animal was somewhat toxic as the result of this large dose and after about 5 hours became quite sleepy. Sacrificed at 12 hours the fetal urine in one bladder showed what was thought to be a trace of ferric iron but the test was questionable. The amniotic fluid was frankly negative. However it seems probable that a very small amount of ferric iron had passed to the fetal circulation and had been excreted as such by the fetal kidneys.

The results obtained when urethane was used as the anesthetic were entirely comparable to those found in experiments performed on animals under ether anesthesia, or on decerebrate animals, as described in the first paper of this series (5).

Discussion. It seems evident from the experiments reported above that the precipitation of the Prussian blue granules indicates the course of the sodium ferrocyanide and the iron ammonium citrate through the placental tissues, and that the latter salt under consideration is in some way arrested or changed during this passage so that it does not enter

Digitized by Google

the fetal circulation in such a form that it can be detected by the reactions used. Further it seems certain that there is some precipitation of the Prussian blue in the living tissue, because such an intra-vitam formation of the characteristic blue granules was not accompanied by any findings indicative of a moribund condition of the cells. If one thinks of this reaction as resulting from the intact molecules of the two salts by the intermediation of an acid, the question arises as to the possibility of acid formation in the living cell; and these cells are unquestionably still alive even though they may be injured to a considerable extent. Whether enough acid can be produced in protoplasm as the result of injury to cause such a precipitation without killing the cell is most difficult to answer. It hardly seems possible in the present case because distribution of the Prussian blue granules was so general throughout the placenta that had the cells been dead the entire placenta must have ceased functioning. It was interesting to find that the death of the fetus did not seem to increase the precipitation of the Prussian blue. On the whole the explanation which seems most logical is that the chorionic ectoderm has the power of altering the chemical structure of this compound, and that one stage in this process results in the change of the iron ammonium citrate in such a way that a compound is formed which would combine with the sodium ferrocyanide and form Prussian blue. Harvey and Bensley (10) have suggested that ammonia might be removed from iron ammonium citrate in the blood stream and the resulting ferric citrate be free to combine with the cyanide; perhaps the reaction taking place in the placenta is similar to this. If this hypothesis were true then longer exposure of animals that had received a single dose given at the beginning of the experiment should show increasing amounts of the Prussian blue formed during life. This has been found to be the case. Again it must be asked what prevents the remaining citrate which is evidently free in the blood stream and unchanged in the ectoderm, from passing to the fetal blood stream. It seems necessary to postulate two forces, one which stops the citrate in its passage through the placenta, and another that is capable of changing it in such a way that a ferric citrate is formed. It is also possible that still further or perhaps different reactions also take place within the cytoplasm of the chorionic ectoderm.

There is also the possibility that the Prussian blue has been formed in the blood stream and the granules actively phagocytized by the tissues of the placenta. The molecules of Prussian blue are as small as those of trypan blue, but it is well known that those of the former tend

Digitized by Google

to form aggregates which are larger than particles of india ink. It is evident therefore that if flocculation has taken place the Prussian blue would not be phagocytized while if the chorionic ectoderm had access to the freshly precipitated molecules a storage similar to that of trypan blue might result. That this is not the case is indicated by the rapidity with which the Prussian blue reaction takes place (8 to 10 hours), while it requires 2 to 3 days for the storage of trypan blue to take place; by the lack of storing of Prussian blue in the numerous cells elsewhere in the body which do store vital dyes; and finally by the absence of Prussian blue granules in the giant cells which are the most active storehouses of true vital dyes. Whatever may be the explanation of this reaction between the two salts and the formation of Prussian blue within the living cytoplasm of the chorionic ectoderm, it seems certain that it cannot be the cause of the impermeability of that structure to the one salt while the other was able to pass to the fetal circulation. This is further evidenced by the fact that the ferrocyanide could be detected in those animals in which there was the greatest precipitation as well as in those in which there was much less, and there was invariably a marked increase in the amount of Prussian blue found in the chorionic ectoderm after acid fixation. That this reaction is of very great importance can not be doubted, but further investigation will be required to determine its meaning and relation to the activities of the placenta.

There is sufficient evidence to conclude that the change which the iron ammonium citrate evidently undergoes is effected in a definite and given area of the tissues intervening between the maternal and fetal circulations. This localization is of considerable interest because one of the most discussed problems in fetal nutrition is whether the foodstuffs used by the fetus are actually prepared in any part by the placenta, or whether they are derived by diffusion directly from the maternal blood plasma, having been prepared by the maternal tissues. A very large group of workers has supported the idea that the placenta was actively engaged in the metabolism of the fetal foodstuffs, and a few have in addition specifically localized these activities within the placenta itself.

Claude Bernard (3) showed that the maternal part of the rabbit's placenta contained large amounts of glycogen. This was substantiated by Driesen (6) in the human. The fetal part of the placenta has also been found to give a glycogen reaction by Langhans (18), (19), and by Zuntz (38).

Digitized by Google

Ascoli (1) found in a large number of serological experiments on foreign proteins that these could not pass the placenta as such, he also found that the placenta contained proteolytic ferments. From these and other experiments he concluded that the placenta was capable of breaking down proteins, but was unable to decide whether these were attacked in the form of total protein molecules or as some intermediate product.

Hofbauer (12), (14) studied the distribution of fat in the human placenta and found that the part of the syncytium adjacent to the maternal circulation contained no fat droplets but they were present in the basal part of the layer. He compares this finding with the distribution of fat in the intestinal cells during absorption of food and concludes that the two processes are strikingly similar. Further Hofbauer fed animals and pregnant women fat stained with Sudan III and he claims to have found that both maternal and fetal bloods contained the dye but the droplets in the placenta and in the fetal tissues were always unstained. On the basis of his histological findings in the human and these experimental findings, he concludes that fats are broken down in the outer layer of the syncytium and resynthesized to form a fat corresponding to that of the fetus. Hofbauer (16) obtained very similar results in studying the absorption of iron, finding that the iron granules detected by microchemical reactions became more and more evident as the fetal vessels were approached. He also considers (15) that the presence of ferments in the placenta has assisted in substantiating his views as outlined above. Kehrer (17) and Heyman (11) also believe that the processes in the placenta are analogous to those in the mechanism of food absorption of the intestine.

The extent to which ferments are involved in placental interchange is still uncertain. Bergell and Falk (2) conclude that there are sufficient enzymes in placental tissues to account for the albumin requirements of the fetus. These enzymes they consider as unquestionably intra-cellular. And they conclude that the placenta is a metabolistic organ, regulating the fetal nourishment, and that it is more analogous in function to the liver than to the intestine. Frank (8) on the contrary after a careful study of placental and fetal ferments and enzymes comes to the conclusion that his results afford no support to the view that the placenta acts as an organ of digestion for the fetus.

During the last four or five years a large number of communications has brought important additional information to assist in analyzing the question of placental transfer. These papers have dealt with the

Digitized by Google

chemical analysis of the bloods of mother and fetus; modern methods having been developed which permitted the determination of a variety of metabolic substances and excretory products in very small amounts of blood. Slemons and Bogert (29) have established that the maternal and fetal bloods have approximately the same content in uric acid, Slemons and Morriss (30) have obtained similar results in comparative studies of the urea and non-protein nitrogen, in the two bloods, and Plass (25), (26) found an equal content in creatine and creatinin. These observers conclude that the exactly similar content of the fetal and maternal bloods indicates that the excretory products pass the placenta by diffusion. Morriss (21) found that the blood sugar was slightly higher in the maternal than in the fetal bloods; this he interprets as meaning a rapid storage or metabolism of sugar by the fetus and does not think it indicates any regulatory mechanism in the placenta. Morse (22) has found that the amino-acid nitrogen is higher in the fetal than in the maternal blood serum. To explain this he assumes a capability of the placenta to "absorb" the amino-acids, a mechanism similar to that suggested by Van Slyke (33), (34), (35) in the case of the removal of amino-acids from the blood stream by the tissues. From this evidence which indicates a one-sided permeability he is forced to postulate a mechanism in the placenta for retaining the amino-acids in the fetal circulation.

Slemons and Stander (31) found that the maternal blood contained a much larger amount of lipoids and fats than the fetal, and Mendel and Daniels (20) found that stained fats would not pass from mother to fetus in the case of pregnant rats. Slemons (27), (28), from these two groups of experiments, concludes that the fats and allied substances do not pass the placenta, and that the fetus constructs its own fats from the carbohydrates supplied it by the mother. The view that the fat molecule as such does not pass the placenta has been generally accepted. Oshima (24) in particular has confirmed this by ultramicroscopic studies on the fat content of maternal and fetal bloods. But that some allied substances can traverse the placenta is indicated by the work of Hofbauer (13) and of Thiemich (32). They fed cocoa-fat to pregnant animals and found fatty acids characteristic of this fat in the fetus. Finally with reference to the transfer of iron practically all the evidence still rests on the microchemical observations. The present status of the question is well stated by Slemons (27) as follows:

With regard to iron it is impossible, at present, to affirm what arrangements are made for its transportation through the placenta. This intricate and unsolved

Digitized by Google

problem occupies a unique position among the factors of fetal nutrition. Stored in the newly born infant there is a large quantity of iron, so large, indeed, that the quantity is proportionately much greater than in the adult. The purpose of this storage in the newborn, Bunge believes, is to compensate for the inadequate amount of iron in human milk.

These various observations have, I think, gone far toward establishing the importance of diffusion in the passage of certain substances particularly the excretory substances of the fetus; but on the other hand the results regarding sugar, amino-acids, fats and iron tend to suggest a regulatory mechanism in the placenta itself. As we have seen, the work of Edelstein and Ylppö (7) indicates a regulatory mechanism for salts, while Cohnstein and Zuntz (4) come to the opposite conclusion.

Nicloux (23) found that alcohol and ether were governed in their transmission through the placenta by the laws of osmosis and diffusion, and from these observations he decided that all diffusible substances including true solutions of soluble salts would be governed by the same laws. In that part of his conclusion which is based upon his own observations there is no possibility of adverse criticism, but in the extension of his theories to include those salts that had only been reported on by others, the conclusion is made too general.

Kehrer (17) concludes that all serum-salts which are not combined with albumin and protein pass through the placenta easily and obtain entrance to the fetal blood. This transfer he believes is controlled by osmosis and diffusion alone. The question of what he means by the combination of salts with proteins is very interesting because it is conceivable that any and every salt may enter into some kind of combination with some larger molecule of the living system. It is very probable that any salt molecule which could diffuse freely into a cell would leave it equally as readily unless some change had taken place in its physicochemical state after it had gained entrance to the cytoplasm. From this brief review of the conflicting views on salt transfer it is evident that a decision can not yet be made as to whether osmosis and diffusion represent the dominant forces engaged in the transfer of those soluble salts which are normally present in the blood plasma.

The forces of osmosis and diffusion seem inadequate to explain the reaction of the protoplasm of the placental cells and syncytium to many colloids. Goldmann (9) and Wislocki (37) have shown that the placenta is practically impermeable to trypan blue and other acid-azo-dyes, while these dyes are stored within the protoplasm. Why do these molecules, too large to "pass through the placenta," pass through one

Digitized by Google

side of the lining substance and not through the other? There must be either a difference in the permeability of the two sides of the placental tissue or else the dye must meet with some chemical or physico-chemical reaction within the living cytoplasm which prohibits its further progress.

The conflicting reports which have been briefly reviewed above, and the results so far reported in this series only furnish sufficient basis for a working hypothesis regarding the mechanism of placental interchange. This theory may be expressed in terms of a reclassification which is dependent upon specific reactions of the placenta to specific substances. Three groups may be indicated: *a*, Those substances which are diffusible and which meet with no mechanism in the placenta capable of acting on them; these pass by diffusion from mother to fetus, or in the reverse direction without any mediation on the part of the placenta. This group contains most of the excretory products of the fetus, and large numbers of foreign substances, many of which are highly toxic. *b*, A group of substances to which the maternal or fetal surfaces of the placental barrier are impermeable. Here may be grouped the formed elements which are normally present in the circulation and such foreign substances as insoluble salts (e.g., barium sulphate) and foreign particulate matter such as india ink, cinnabar and bacteria. *c*, Certain substances which meet a definite preformed regulatory mechanism in the placenta. At present this group must include most of those substances which are designed for the fetal metabolism and certain important inorganic salts, especially those containing iron.

This hypothesis emphasizes the idea that the placenta is an apparatus to insure the receipt and retention of sufficient materials for fetal metabolism, and that this is accomplished by the development of certain specific reactions which are probably entirely separate entities in their physico-chemical nature. That the iron ammonium citrate undergoes some change within the chorionic syncytium is indicated by the results of the experiments reported here. But there is very little evidence as to what the nature of the reaction may be save that at some stage a compound is formed that reacts with sodium ferrocyanide to form Prussian blue; with regard to the experiments in which iron ammonium citrate was used alone the evidence does not seem to be sufficient to authorize the conclusion that an excess beyond the normal capacity of the placenta would diffuse through into the fetal circulation, but is only suggestive of that. However even if such an hypothesis be warranted there is still the evident fact that the mechanism is capable of

Digitized by Google

dealing with an almost unbelievable amount of the salt, so much in fact that experimentally it does not seem possible to examine it because the limit of the amount of the salt that the animal can stand seems to be about equal to that amount which the placenta is capable of handling.

CONCLUSION

The results reported here suggest that the iron ammonium citrate meets a specific regulatory mechanism which is capable of changing quite large amounts of this salt. It seems probable that this mechanism is normally concerned with the control of the passage of iron containing substances, the decomposition of which is necessary for the preparation of iron for fetal use and storage. That this conclusion can only be suggestive and not absolute is clear, but it is certain that the chorionic ectoderm in the cat does react differently to two salts whose diffusion rates are not greatly dissimilar when measured by their reaction to other living membranes.

BIBLIOGRAPHY

(1) Ascoli: Zeitschr. f. phys. Chem., 1902, xxxvi, 498.
(2) Bergell and Falk: Münch. med. Wochenschr., 1908, lv, 2217.
(3) Bernard: Journ. de la Physiol. de l'Homme, 1859, ii, 31; Abst. Amer. Med. Monthly, 1859, xi.
(4) Cohnstein and Zuntz: Arch. f. d. gesammt. Physiol., 1888, xlii, 342.
(5) Cunningham: This Journal, 1920, liii, 439.
(6) Driessen: Arch. f. Gynäk., 1907, lxxxii, 278.
(7) Edelstein and Ylppö: Zeitschr. f. Kinderheilk., 1920, xxvii, 79.
(8) Frank: Surg. Gynec. and Obst., 1912, xv, 558; Trans. Amer. Gynec. Soc., 1912, xxxvii, 455.
(9) Goldmann: Aussere und innere Sekretion im Lichte der Vitalen Farbung, Tübingen, 1912.
(10) Harvey and Bensley: Biol. Bull., 1912, xxiii, 225.
(11) Heyman: Fol. Haemat., 1906, iii, 7–18; 71.
(12) Hofbauer: Biologie der menschlichen Plazenta, Wien, 1905.
(13) Hofbauer: Samml. Klin. Fortr., 1907, S. xvi, N. 166, 1930.
(14) Hofbauer: Anat. Anz., 1904, xxv, Erghanschft. 99.
(15) Hofbauer: Wien. klin. Wochenschr., 1904, xvii, 939.
(16) Hofbauer: Zeitschr. f. Phys. Chem., 1903–4, xl, 240.
(17) Kehrer: Würzburger Abhandl., 1907, vii, 17.
(18) Langhans: Arch. f. Anat. u. Phys., Anat. Abth., 1877, 188.
(19) Langhans: Arch. f. path. Anat., 1890, cxx, 28.
(20) Mendel and Daniels: Journ. Biol. Chem., 1912–13, xiii, 140.
(21) Morriss: Johns Hopkins Hosp. Bull., 1917, xxviii, 140.
(22) Morse: Johns Hopkins Hosp. Bull., 1917, xxviii, 199.
(23) Nicloux: Obstétrique, 1909, n. s. ii, 840.

Digitized by Google

(24) Oshima: Zentralbl. f. Physiol., 1907, xxi, 297.
(25) Plass: Johns Hopkins Hosp. Bull., 1917, xxviii, 137.
(26) Plass: Johns Hopkins Hosp. Bull., 1917, xxviii, 297.
(27) Slemons: Nutrition of the foetus, 1919, Yale Press.
(28) Slemons: Proc. Conn. Med. Soc., 1916, cxxiv, 100.
(29) Slemons and Bogert: Journ. Biol. Chem., 1917, xxxii, 63.
(30) Slemons and Morriss: Johns Hopkins Hosp. Bull., 1916, xxvii, 343.
(31) Slemons and Stander: Trans. Amer. Soc. for Advancement of Clinical Investigation, 1918.
(32) Thiemich: Jahrb. f. Kinderheilk., 1905, lxi, 174.
(33) Van Slyke: Journ. Biol. Chem., 1913–14, xvi, 213.
(34) Van Slyke: Journ. Biol. Chem., 1913–14, xvi, 187.
(35) Van Slyke and Meyer: Journ. Biol. Chem., 1913–14, 197.
(36) Wislocki: Contrib. to Embryol., no. 51, Vol. xi, Carneg. Inst. of Wash. Publ. no. 274, 1920.
(37) Wislocki: Anat. Record, 1921, xxi, 29.
(38) Zuntz: Ergebn. der Physiol., 1908, vii, 403

Digitized by Google

STUDIES IN EXPERIMENTAL TRAUMATIC SHOCK

VI. The Liberation of Epinephrin in Traumatic Shock

D. RAPPORT

From the Laboratory of Physiology in the Harvard Medical School

Received for publication January 10, 1922

There is evidence that during the development of shock there is a general over-activity of the sympathetic division of the autonomic system. Thus, there is acceleration of the heart, dilatation of the pupils, and a generalized vasoconstriction. This latter factor has recently received considerable attention and has been emphasized as an important conserving mechanism. Guthrie (1) has observed that cutting the nerve supply of a limb in a normal animal would result in an increase of blood flow through that limb of 22 per cent, whereas performing the same maneuver on a shocked animal resulted in a rise of blood flow amounting to 76 per cent. Erlanger, Gesell and Gasser (2) found that in shock produced by handling the abdominal viscera there was marked constriction of the limb vessels as the blood pressure dropped, and that not until the pressure had reached 50 mm. Hg. did the vessels of the limb dilate, as determined by their inflow method. Cattell (3) has noted a similar vasoconstriction in the development of traumatic shock. In his experiments, vasodilatation did not occur until, on the average, the blood pressure had been reduced to 65 mm. Hg.

Accompanying this vasoconstriction, and particularly in view of the symptoms of general sympathetic stimulation, it would be not unreasonable to postulate the possibility of an increased activity of the adrenal glands, acting either as an ungovernable and deleterious factor in the development of shock, or as a secondary conserving mechanism, or, conceivably, as an incidental concomitant, without special significance in this connection.

Whether or not such an increased activity of the adrenals occurs has been in dispute. Bedford and Jackson (4) and Bedford (5) published observations tending to show that in shock produced by handling of the intestine, hemorrhage and occlusion of the inferior vena cava, there

Digitized by Google

is an increased amount of epinephrin in the caval blood at a point opposite the entrance of the lumbo-adrenal veins. Dogs were used, and the method involved opening of the abdomen and a certain amount of preliminary manipulation of the abdominal contents before the supposed control specimens were obtained. The blood was tested for epinephrin by means of rabbit intestinal "strips." The conclusions arrived at by Bedford were: *a*, Increased quantities of epinephrin are thrown into the blood during conditions of low blood pressure and shock. *b*, This is due to hyperactivity—not to depletion of the glands. *c*, The quantity of epinephric material in the blood increases only after a somewhat prolonged continuation of the conditions leading to shock. *d*, The quantity of epinephric material in the blood increases with the prolongation of the period of low blood pressure and shock. *e*, This increased output of epinephrin into the blood may be a last effort on the part of the organism to resist the forces that are tending toward a fatal degree of low blood pressure.

Stewart and Rogoff (6) repeated this work, using essentially similar methods, but were unable to obtain any evidence of increased liberation of epinephrin per unit of time. Shock was produced by intestinal manipulation, hemorrhage, and "peptone" injections, and blood from the "cava pocket" was assayed by means of rabbit intestine and uterus segments. These authors criticise the work of Bedford on the ground that he did not estimate quantitatively the amount of epinephrin per unit of time. As a matter of fact, however, he appears to have taken into account the rate of flow from the lumbo-adrenal veins and, allowing for differences in the rate, there is still evidence in his published figures of increased liberation of epinephrin in the conditions of shock produced in his experiments.

In view of the above conflicting results, and in view of the possible significance of epinephrin over- or under-production as a shock inducing factor, the experiments that I report were undertaken.

Method. Cats were used throughout, and they were anesthetized with ether. Thirteen experiments were performed, of which four were controls, in which the adrenals had been removed prior to the induction of shock. The latter was accomplished by crushing the thigh muscles of one leg. In the majority of cases the sciatic and femoral nerve trunks were cut cephalad to the region of crushing, so as to rule out, as far as possible, the question of reflex nervous stimulation of the adrenal glands. It may be said, in passing, that no striking difference was observed in the two types of experiments and, consequently, any posi-

Digitized by Google

tive results obtained were not the effect of repeated passage of impulses centrally along the large nerve trunks of the leg.

The heart, isolated from its extrinsic nervous mechanism by cutting the vagi in the neck and removing the stellate ganglia, was employed as an indicator of the liberation of epinephrin into the blood stream. This method has been described by Cannon (7). The stellate ganglia were removed through an opening between the first and second ribs, under artificial respiration. Blood pressure and heart rate were recorded by a cannula in the carotid artery, connected to a mercury manometer. A tracheal cannula was held in place by a tie that included the inferior thyroid veins. It was important to keep the temperature as nearly constant as possible. This was accomplished with sufficient exactness to prevent this factor from having appreciable influence on the results.

Experimental observations. Of nine experiments, excluding the controls, five showed a definite rise of rate as the blood pressure was falling, indicating increased liberation of epinephrin. Three showed either no change or a slight drop in the rate, usually gradual. In one experiment, there was an initial rise in rate immediately after crushing the muscles, due possibly to nervous stimulation, as the leg nerves had not been cut. Following this, the rate dropped to a point slightly below the original rate, but 42 minutes after the injury the rate began to rise and continued elevated until death, 70 minutes later.

It is interesting to note that, in one case (cat 10, group I), after crushing and massaging the muscles of the thigh, there was a gradual increase in the heart rate (196 to 212), while the fall in blood pressure was slight (122 to 100). Moreover, the blood pressure at this time could not be said to have reached a "shock level." Under the usual experimental conditions, we should expect to find with this slight drop in blood pressure either no change in the heart rate, or a slight fall. The fact that the heart rate was increased indicates the onset of a condition of shock, to which the blood pressure curve would have given no definite clue. This is in accord with the observations of Gesell (8) who noted that the development of shock might be indicated by the reduction in the volume flow of blood through the submaxillary gland as a result of vasoconstriction—when the drop in blood pressure had not yet assumed significant proportions.

It will be well, perhaps, to analyze briefly the individual experiments.

Digitized by Google

Group I. In which the rate was elevated during the development of shock. Five cases.

Cat 2, ♀, 2.9 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
1:35	38.1	143	228
1:40	38.1	152	228
1:45	38.1	155	228
1:45–48	Crushed muscles of left thigh		
1:48	38.1	69	228
2:00	38.1	118	237
2:05	38.2	94	248
2:15	38.1	84	242
2:25	37.6	79	234
2:35	37.8	64	231
2:45	38.0	52	220
2:50	37.2	48	214
3:00	37.8	41	224
3:05	38.0	40	222
3:10	38.1	40	221
3:15	38.0	24	202
3:25	37.9	20	184
Death of animal			

Average rate before injury, 228. Highest rise 20 beats, 17 minutes after injury. Rate elevated for 47 minutes, following injury.

Cat 5, ♀, 2.6 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:35	37.9	124	190
2:40	37.9	120	184
2:45	37.9	116	183
2:45–47	Crushed muscles of left thigh		
2:47	37.8	92	200
2:55	37.8	81	189
3:00	38.0	83	193
3:10	38.0	83	197
3:20	38.0	80	204
3:30	38.0	78	196
3.40	37.7	56	187
3:50	37.8	47	188
3:55	37.7	47	189
4:05	37.9	49	194
4:15	38.2	48	194
4:30	38.1	50	193

Average rate before injury 186. Highest rise 18 beats, 33 minutes after injury. Rate elevated for 43 minutes, following injury.

Digitized by Google

Cat 8, ♀, 3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:30	37.6	135	192
10:35	37.6	132	191
10:40	37.7	135	191
10:43–45	Crushed muscles of right thigh		
10:45	37.7	80	204
10:55	37.6	102	212
11:05	37.6	99	214
11:15	37.8	96	212
11:25	37.6	98	213
11:35	37.6	90	208
11:45	37.7	80	208
12:00	37.6	74	206
12:10	37.6	68	196
12:20	37.6	63	190
12:30	37.6	54	187
12:40	37.6	49	189
12:50	37.5	42	184
Death			

Average rate before injury 191. Highest rise 25 beats, 20 minutes after injury. Rate elevated for 85 minutes, following injury.

Cat 9, ♀, 3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Left sciatic and left femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:30	36.8	129	208
2:35	36.8	132	202
2:40	37.0	130	202
2:41–43	Crushed muscles of left thigh		
2:43	37.0	97	220
2:50	37.0	105	216
3:00	37.1	99	224
3:10	37.0	89	230
3:20	37.0	78	226
3:30	36.8	63	214
3:45	36.5	52	198
4:00	36.8	58	208
Death			

Average rate before injury 204. Highest rise 24 beats, 27 minutes after injury. In this animal there was a slight premortem rise, 10 beats above the rate of the previous observation.

Digitized by Google

Cat 10, 2.5 K. Ether anesthesia. Vagi cut in neck and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:50	37.8	140	196
10:55	37.8	140	198
11:00	37.8	140	196
11:03–05	Crushed muscles of right thigh		
11:05	37.8	96	198
11:10	37.8	135	198
11:20	38.0	122	196
11:24–25	Massaged muscles of crushed thigh		
11:25	38.0	112	204
11:30	38.0	120	200
11:35	38.0	115	208
11.45	38.1	108	212
11:50	38.1	105	212
12:00	38.1	100	208
12:10	38.0	88	214
12:20	37.9	82	212
12:25	37.8	80	210
12:40	37.6	83	208
12:50	37.7	77	198
1:00	37.7	74	194
1:15	37.9	45	204
Death			

Average rate before injury 197. Highest rise 17 beats, 65 minutes after injury. Rate elevated for 105 minutes, following injury. There was in this cat, as in cat 9, a slight premortem rise of 10 beats. In cat 9 this was associated with a slight rise in blood pressure, which was absent in cat 10. I can offer no logical explanation for this phenomenon.

Cat 1, group II, was the only case in which there did not appear to be any indication of over-activity of the adrenals shortly after injury. In this instance the pressure dropped after injury from an original level of 108 mm. to 65 mm., which is probably below the critical level for adequate nourishment of the heart. The recovery on the next reading was slight—74 mm. Hg., and thereafter the pressure never rose above this dangerous level. It is conceivable that the sharp and continued drop in pressure was injurious to cardiac tissue. Thus in a number of cases reported by Stewart and Rogoff (9, cases 443, 447, 448) a progressive fall of blood pressure below 80 mm. Hg. was attended by a more or less progressive fall in the heart rate. Similar observations have been made by Cannon and Smith. Probably in the presence of very low pressure

Digitized by Google

Group II. In which the rate was not elevated until shock was fully established. One case.

Cat 1, ♀, 2.4 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:20	37.8	105	194
10:25	37.8	109	190
10.30	37.8	108	190
10:30–33	Crushed muscles of left thigh		
10:33	37.8	65	212
10:40	37.8	74	193
10:45	37.8	73	186
10:55	37.8	72	175
11:00	37.4	68	178
11:05	37.4	63	182
11:10	37.4	62	183
11:15	37.7	56	188
11:25	38.0	51	198
11:35	38.2	53	206
11:50	38.4	54	203
12:05	38.1	54	209
12:15	37.9	38	204
12:20	37.8	36	210
12:25	37.8	38	216
Death			

epinephrin cannot have the degree of effect that it has when the pressure is not so much reduced. The harmful influence of a lessened blood flow is doubtless due both to oxygen want and to excess of carbon dioxide and perhaps other metabolites. Patterson (10) found a direct antagonism between the action of CO_2 and epinephrin on the rate of beat of the isolated heart—CO_2 slowing the rate, epinephrin making it more rapid. The combination of epinephrin and CO_2 had an effect that was somewhere between the effect of each separately, and in one experiment the rate was slower than the normal rate of the heart. In such a heart as we have in cat 1, we may conceive of asphyxia and the products of asphyxia as contending with an increased production of epinephrin for mastery over the heart, the one tending to decrease the rate of the heart, the other to increase it. It will be noted that no analogous condition—as regards blood pressure—was present in any of the five cases in which early rises in rate were observed. In cat 3, where there was no rise in rate, somewhat similar conditions prevailed.

Digitized by Google

Group III. In which the rate was not elevated during the development of shock. Three cases.

Cat 3, 4.1 K. Ether anesthesia. Vagi cut and stellate ganglia removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
10:35	37.0	147	189
10:40	37.0	147	189
10:45	37.0	130	193
10:50	37.0	133	192
10:55–59	Crushed muscles of left thigh		
10:59	37.0	60	186
11:10	37.0	63	192
11:15	37.1	75	187
11.20	37.2	76	190
11:25	37.2	77	190
11.30	37.2	75	182
11:35	37.2	57	176
11:40	37.2	49	173
11:50	37.2	39	174
12:00	37.1	25	172
12:05	37.1	15	150
Death			

Average rate before injury 191. Very slight change in rate for 26 minutes, then a drop of 16 beats. The blood pressure conditions in this cat were somewhat analogous to those of cat 1. In this case, however, the drop was greater—about 67 mm. Hg. The pressure was from the start at a dangerously low level after the injury, and it is possible that the heart had lost some of its power of reacting to epinephrin.

In the three cases of group III, there was no evidence of increased activity of the adrenal glands. In two of them (cats 12 and 13), I am not convinced that this can be adequately accounted for. It might be argued that were it not for increased secretion by the glands in these cases, the rate would have been much lower than was actually the case. It will be seen, however, on examination of the data obtained from control cats, whose adrenals had been removed before crushing the limb, that, save in one instance, there was no marked drop in the heart rate, in spite of the fact that the blood pressure was in comparable cases no lower than at corresponding points of the development of shock in cats 12 and 13, whose adrenals were intact.

Digitized by Google

Cat 12, 3.3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:20	35.7	128	172
2:25	35.7	133	168
2:30	35.7	136	168
2:35–37	Crushed muscles of right thigh		
2:37	35.7	85	168
2:45	35.8	94	172
2:50	35.8	89	172
2:55	35.8	85	172
3:05	35.7	74	172
3:15	35.7	63	168
3:25	35.4	59	164
3:35	35.4	57	162
3:45	35.3	56	160
4:05	35.8	59	166
4:20	35.8	59	164

There was practically no change in rate, despite a fair pressure for some time after the injury.

Cat 13, 3.5 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and right femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:40	38.0	127	196
2:45	38.0	123	196
2:50	38.0	122	196
2:52–53	Crushed muscles of right thigh		
2:53	37.9	100	192
3:00	38.2	86	194
3:05	38.3	84	192
3:10	38.4	81	196
3:15	38.5	77	200
3:25	38.4	74	200
3:35	38.2	71	200
3:45	38.2	71	196
4:00	38.1	50	190
4:15	38.1	44	184
Death			

There was a slight rise of temperature in this case. Allowing for this, the rate remained practically unchanged.

Digitized by Google

Group IV. In which the adrenal glands were removed prior to the induction of shock.

Cat 4. Ether anesthesia. Vagi cut and stellate ganglia removed. Adrenals removed

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
11:10	Adrenals out		
11:35	37.0	131	194
11:40	37.0	120	198
11:45	37.1	125	189
11:45–49	Crushed muscles of left thigh		
11:49	36.8	78	170
11:55	36.6	94	161
12:00	36.6	89	162
12:05	36.6	95	160
12:10	36.7	91	161
12:15	37.1	92	166
12:30	37.5	71	176
12:35	37.6	66	176
12:40	37.6	52	173
12:45	37.6	50	173
12:50	37.7	45	170
12:55	37.4	39	164
1:00	37.4	39	160
1:05	37.3	36	158
Death			

In this animal a fairly high initial rate was lowered at once, about 16 beats after the muscles were crushed, and remained at about this low level during the development of shock. During the first 25 minutes after injury, the rate was, on the average, 27 beats below the original level.

Cat 6. 2.9 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Left sciatic and left femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:25	Right adrenal gland removed		
2:40	Left adrenal gland removed		
2:55	34.7	96	134
3:00	34.7	93	136
3:05	34.7	93	136
3:07–08	Crushed muscles of left thigh		
3:10	34.7	80	138
3:15	34.7	78	138
3:25	34.7	74	136
3:35	34.8	70	135
3:45	35.0	70	135
3:55	35.0	65	136
4:05	34.8	63	134
4:15	34.7	60	132
4.25	34.8	50	132
4:40	35.2	54	132
4:55	35.2	52	132
5:10	35.0	51	130

A very low original rate, with practically no change after injury.

Digitized by Google

Cat 7, 2.9 K. Vagi cut and stellate ganglia removed. Left sciatic and left femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:10	Left adrenal gland removed		
2:25	Right adrenal gland removed		
2:35	37.8	137	168
2:45	38.0	143	170
2:50	38.0	138	170
2:53–56	Crushed muscles of left thigh		
3:00	38.0	103	163
3:05	38.0	94	162
3:15	38.0	92	161
3:25	38.0	86	165
3:35	38.0	84	166
3:45	38.0	68	164
3:55	38.0	67	164
4:05	38.1	68	166
4:15	38.0	68	164
4:25	38.1	72	163
4:35	37.9	79	163
4:40	Gentle massage of crushed muscles		
4:45	38.3	70	160
4:55	38.1	69	156
5:10	37.9	65	156

Shock developed slowly in this animal, but there was no evidence of increased heart rate even when the blood pressure was above 90, after tissue injury.

Cat 14, 3 K. Ether anesthesia. Vagi cut and stellate ganglia removed. Right sciatic and femoral nerves cut

TIME	TEMPERATURE	BLOOD PRESSURE	HEART RATE
2:00	Right adrenal gland removed		
2:15	Left adrenal gland removed		
2:30	37.0	118	164
2:35	37.0	116	163
2:40	37.0	118	166
2:42–43	Crushed muscles of right thigh		
2:43	37.0	81	164
2:55	37.1	100	152
3:05	37.0	100	154
3:15	37.0	97	158
3:25	37.0	90	154
3:35	37.0	84	154
3:45	37.1	80	156
4:00	37.0	78	154
4:10	36.8	76	156
4:20	36.9	70	152
4:30	37.0	65	150
4:40	37.0	60	146
4:50	37.0	54	144
5:00	36.8	46	143

In this cat, also, there was no rise in heart rate after crushing the thigh muscles, even when the blood pressure was above 90. The rate tended to be about 8 or 10 beats lower than the original rate for about 80 minutes after injury.

Digitized by Google

Of the control animals, cat 6 had what might be described as almost a basal rate. In other words, the possibilities of a drop of rate, as a result of the gradual decrease in blood pressure, were much reduced. It is probable that with the heart beating at this rate, its nutritional needs are not so great as in a faster beating heart doing just as much external work, and that it is not so susceptible to changes in blood supply below the usual critical pressure. In cats 7 and 14, also, the heart rate was lower than is usually the case in animals with adrenals intact. In cat 4, where the original rate was fairly high (average 193), tissue injury was followed by a marked drop in heart rate, associated with the lowered blood pressure, suggesting that the above hypothesis has a basis in fact.

Discussion. Of nine cases, there was in five a definite increase in the rate of the isolated heart with a falling blood pressure, early in the course of the development of traumatic shock. This increased rate appeared quickly, often in the first observation after crushing the thigh muscles. The continued elevation in rate can not be due to continued stimulation of nerve endings in the injured region, resulting in reflex discharge from the adrenals, for the change appears to as marked a degree in cases where the chief afferent paths above the region have been cut, as where they have not.

It must be emphasized that, while as a result of these experiments I take issue with Bedford when he states that there is an increase in epinephrin liberation only after a prolonged continuation of the conditions leading to shock, I am not prepared to deny his opinion that the quantity of epinephric material in the blood increases with the prolongation of the period of low blood pressure and shock, for the method I used has this limitation, that when the blood pressure becomes low—let us say 50–60 mm. Hg.—the tendency of the heart rate, as mentioned above is to drop, because the heart is being insufficiently nourished. Under these circumstances, the rate of the isolated heart would be the resultant of opposite factors, increased epinephrin liberation tending to accelerate it, and the decreased blood supply tending to make it slower. So, in the experiments described, it is possible that in the later stages of shock there was an increased discharge of epinephrin into the blood stream, which the method I used was unable to detect. Of course, the tendency of the heart rate to become slower as the pressure falls renders especially significant the actual *increase* of rate which was manifested in the foregoing experiments. In other words, the experimental conditions were opposed to the positive result which was obtained.

Digitized by Google

One case, of the remaining four, also showed an increase in heart rate after injury, but in this case the rise did not appear until shock was fully developed, as indicated by the blood pressure. This is the only case that gave results similar to Bedford's observations. I have already discussed a possible reason for the delay in the appearance of the rise in this case. It is remarkable that it should have appeared at all in view of the then long continued low pressure.

Relation of epinephrin liberation to the production of shock. If, in most cases, there is this increased liberation of epinephrin into the blood stream during the development of traumatic shock, the question arises as to its significance.

It has been postulated that shock is the result of exhaustion of the adrenal glands. The evidence for this is not convincing. Corbett (11) has stated that anything that depletes epinephrin favors the development of shock. His experiments, of which the details are omitted, tended to show that anesthesia, fright and trauma decrease the epinephrin content of the adrenal glands. Earlier, Bainbridge and Parkinson (12) and Parkinson (13) were unable to demonstrate chromaffin substance in the adrenal medulla in death from post-operative shock and other acute conditions, and asserted that in such acute conditions, associated with low blood pressure, the adrenal glands yield up their store of epinephrin. That this is a fact Short (14) has denied, his examination of adrenals after death from shock betraying no marked difference from controls.

But even assuming that the former observations are correct, they throw no light upon the production of shock, for they furnish no proof that depletion of the epinephrin store in the adrenals did not occur after shock had developed. rather than before.

Moreover, it is obvious that, according to an "exhaustion" hypothesis, even a normal secretion of epinephrin could not exist in the presence of shock. In the experiments which I report, increased activity of the adrenals may be observed in cases where shock was severe enough to be followed shortly by death, and where the blood pressure was at a dangerously low level. Thus, in cat 2 the rate was still elevated with the blood pressure at 64 mm., a drop of 86 mm. from the original level; in cat 8, at 68 mm., a drop of 66 mm.; and in cat 9, with the blood pressure at 63 mm., a drop of 67 mm.—definite evidence that shock was developing in association with an increased secretion of epinephrin.

But is this increased secretion, perhaps, the cause of the development of shock? Such a suggestion has been advanced. Thus, Bainbridge and Trevan (15) injected large amounts of epinephrin, keeping the blood

Digitized by Google

pressure at a high level, and Erlanger and Gasser (16) repeated these experiments, infusing into the femoral vein 6 to 11 cc. of a 1:1000 solution of adrenalin chloride for a period of 21 to 29 minutes. These two series of experiments gave similar results, namely, a marked drop in arterial pressure and symptoms of shock. But the conditions were sufficiently artificial to make them difficult of comparison with the actual conditions of traumatic shock in an animal whose supply of epinephrin is the result of his own manufacture.

In my experiments the highest increase of rate observed was no greater than 25 beats. In the course of recent experiments conducted by Cannon and myself, we have found that the denervated heart reacts quantitatively to the infusion of adrenalin chloride. The reaction is more exact when the animal is "reduced"—that is, when the carotid and subclavian arteries and the aorta below the renal arteries are tied off, and the mesenteric nerves are cut—but even when this procedure is not carried out, the increase of heart rate corresponding to the infusion of definite quantities of adrenalin chloride at a given rate may vary in different animals within comparatively narrow limits. Thus, in one experiment, adrenalin chloride, 1:100,000, injected at the rate of 0.002 mgm. per kilo per minute, produced a rise of 24 beats per minute in the heart rate; and in another, infusion of adrenalin at the rate of 0.0024 mgm. per kilo per minute resulted in a similar rise. Erlanger and Gasser, using dogs, injected very large amounts of adrenalin chloride, far more than would be secreted by the adrenal glands under any circumstances. In one experiment the rate was about 0.04 mgm. per kilo per minute, whereas, in my extreme case, the rate of production of epinephrin was probably in the neighborhood of 0.002 mgm. per kilo per minute. The rate of infusion by Erlanger and Gasser, therefore, can hardly be said to be analogous to the production of epinephrin in the body.

Moreover, Henderson, Prince and Haggard (17) infused, continuously or intermittently, 1:10,000 adrenalin chloride into the femoral vein at a rate of from 0.5 cc. to 1.0 cc. per minute, maintaining the blood pressure at a very high level for a period of from ½ hour to 2 hours, and then, on discontinuing the infusion, found that no symptoms of shock developed. There is, then, no real proof that an increased production of epinephrin is an essential factor in the development of shock.

That it is not an indispensable factor is obvious from the fact that traumatic shock can be produced in the absence of the adrenals or when they give no evidence of hyperactivity, other experimental conditions remaining the same as in such cases where the adrenals are overactive.

Digitized by Google

Cannon has emphasized the idea that in times of stress, hyper-secretion of the adrenal glands accompanies increased activity of the sympathetic division of the autonomic system. In the development of shock such increased sympathetic activity undoubtedly occurs, and one of its most important functions is to produce generalized vasoconstriction in non-vital regions, thus tending to confine the depleted circulation to the organs essential to life. It is possible to invoke a useful function for the over-production of epinephrin in the development of shock, rather than to suppose that the adrenals are running amuck, inducing shock either by hyper-secretion directly, or by exhaustion of their store of epinephrin. They are stimulated to over-activity very soon after the conditions that tend to result in shock are established, and it seems probable that this over-activity, when it occurs, acts as an accessory factor, aiding the sympathetic system in maintaining vasoconstriction, and so may be considered as an additional conservative mechanism, tending to protect the animal against the consequences of shock-inducing influences.

SUMMARY

1. In six out of nine cases reported, there is evidence of hyperactivity of the adrenal glands during the development of traumatic shock.

2. There is no sufficient reason to believe that either over-secretion of the adrenals, or their exhaustion, is a factor in the production of shock.

3. It is probable that over-activity of the glands, in the development of shock, is a conserving factor.

BIBLIOGRAPHY

(1) Guthrie: Journ. Amer. Med. Assoc., 1917, lxix, 1395.
(2) Erlanger, Gesell and Gasser: This Journal, 1919, xlix, 90.
(3) See Cannon: Arch. of Surg., 1922, iv, 1.
(4) Bedford and Jackson: Proc. Soc. Exper. Biol. and Med., 1916, xiii, 85.
(5) Bedford: This Journal, 1917, xliii, 235.
(6) Stewart and Rogoff: This Journal, 1919, xlviii, 22.
(7) Cannon: Science, 1917, xlv, 463.
(8) Gesell: This Journal, 1918, xlvii, 468.
(9) Stewart and Rogoff: This Journal, 1920, lii, 521.
(10) Patterson: Proc. Roy. Soc., London, 1915, lxxxviii, B, 380.
(11) Corbett: Journ. Amer. Med. Assoc., 1915, lxv, 380.
(12) Bainbridge and Parkinson: Lancet, 1907–1, 1296.
(13) Parkinson: Trans. Path. Soc., 1907, lviii, 187.
(14) Short: Lancet, 1914,–1, 731.
(15) Bainbridge and Trevan: Brit. Med. Journ., 1917,–1, 381.
(16) Erlanger and Gasser: This Journal, 1919, xlix, 345.
(17) Henderson, Prince and Haggard: Journ. Amer. Med. Assoc., 1917, lxix, 965.

Digitized by Google

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

IX. Further Evidence of Nervous Control of Thyroid Secretion[1]

W. B. CANNON and P. E. SMITH

From the Laboratories of Physiology in the Harvard Medical School

Received for publication January 16, 1922

In the second paper (1) of this series was summarized the histological and anatomical evidence that the cells of the thyroid gland are innervated by non-medullated fibers arising in cervical sympathetic ganglia. Researches which revealed structural and chemical changes in the gland in consequence of stimulation or severance of nerves in the neck were also reported. Furthermore, we described experiments showing that excitation of the cervical sympathetic induces an action current in the thyroid—a result not seen after vagal stimulation. In the fourth paper (2) Levy presented confirmatory evidence of sympathetic control of thyroid secretion, as shown by increased sensitiveness of vascular responses to repeated standard injections of adrenalin.

The method used by Levy involved pithing the central nervous system to the mid-thorax, a procedure which, of course, excluded any tests of the effects of reflex or asphyxial discharge of sympathetic impulses. Another method permitting such tests was desirable. Since the adrenal medulla secretes continuously under experimental conditions, if the central nervous system is intact, it seemed that Levy's method might be reversed, i.e., that thyroid secretion could render more effective the continuously secreted adrenin. Moreover, it was thought that the heart, isolated from the central nervous system, would probably respond to the combined action of thyroid and adrenal substance. As shown in a previous paper (3), the denervated heart is remarkably stable in its performance and is unaffected by any but

[1] The results here reported were presented to the Society for Experimental Biology and Medicine, February 18, 1920. (See Proc. Soc. Exper. Biol. and Med., 1920, xvii, 88.) It is a pleasure to acknowledge here the support for the research obtained from a fund for thyroid investigation given by Dr. W. N. Bullard.

Digitized by Google

thermal changes and chemical agents brought to it in the blood stream. It manifests characteristic increments of rate when the adrenal medulla (3), (4) or the liver (5) is stimulated. Might it not show another typical change if a thyroid were added to the adrenal effect? Such was the idea at the start. Later, as will be described, it was proved that the thyroid can act directly, without requiring the presence of circulating adrenin.

It may appear strange that an attempt should be made to employ the same organ to test a number of different glands of internal secretion. The heart, however, is a representative muscular structure, continuously active, and working at a rate which is altered with alterations in the rate of metabolism, as, for example, when chemical changes are accelerated by heat (6). Some of the endocrine glands, especially the thyroid, have a pronounced effect on the metabolic rate of the body as a whole. If the heart as a representative organ discloses this effect and is responsive in a typical manner to thyroid stimulation, different from its response to stimulation of other glands, it can obviously serve for testing thyroid activity. And, compared with vascular reactions as a test, it has the advantage of not requiring a destruction of the upper portion of the central nervous system. With it, therefore, conditions known to evoke sympathetic impulses, e.g., afferent stimulation and asphyxia, can be tried, as well as direct excitation of the gland and of peripheral nerves.

Method. In this research the cat has been used for study. Our first experiments were performed under urethane anesthesia. The results were baffling; sometimes suggestive changes occurred, but often no definite effects were evident. Attention to other problems for a number of years had obscured the memory of earlier difficulties of the same kind. In 1916, Cannon and Cattell had reported that in studying the action current of the thyroid gland urethane was unsatisfactory as an anesthetic, and also that deep etherization was capable of abolishing effects that appeared readily when the anesthesia was not so profound (1, p. 62). When these facts were recalled, and light etherization was employed, we at once began to obtain consistent results under conditions to be described. By careful adjustment of the air and the ether vapor entering the trachea, it was usually possible to maintain for long periods a uniform anesthesia, as tested by the wink reflex. A return to urethane in two experiments after repeated successes with ether anesthesia revealed again the depressant influence of that drug.

Digitized by Google

Hoping that we might be able to dispense with anesthesia during the thyroid stimulation, we tried in several instances decerebration. This may prove to be a feasible method, but in the few experiments we tried the blood pressure fell to a low level and prevented a positive result, or the heart rate became so rapid that further acceleration was impossible. We confined ourselves, therefore, to the use of ether, given cautiously and carefully in order to reduce the element of excitement, and maintained at a low tension. In experiments on reflex stimulation the anesthesia was not so deep as to prevent dilatation of the pupil and retraction of the nictitating membrane while the stimulus was being applied.

The tracheal cannula was introduced as low in the neck and with as little disturbance to cervical structures as possible. The opening in the trachea, made at one side in order to avoid the ventral vein which drains the thyroid glands, was so small that after the cannula was pushed in a ligature was not needed to hold it in place.

In isolating the heart the first step, taken even before the trachea was opened, was the severance of both the vagus nerves and the cervical sympathetic strands low in the neck. The object of this early cutting of the sympathetic strands was to protect the thyroids as much as possible from any disturbance incident to operating on the stellate ganglia. To remove the stellates a small opening was made between the first and second ribs on either side, under artificial respiration, and while the ribs were separated by a rib spreader the ganglion was picked up in forceps, its nervous connections were cut with small scissors, and it was brought out entire. The ribs were then tied together (at full inflation of the lungs), the overlying muscles were sewed in two layers, and finally the skin opening was closed. After this operation was completed on each side the animal breathed normally, without artificial aid. Occasionally slight leakage, or failure of the lungs to inflate in all parts when the ribs were being tied, required withdrawing air from the pleural cavity. This was done through a hollow needle.

In isolating the heart from the central nervous system without disturbing the nervous connections of the thyroid gland, as was necessary in experiments on *reflex* and *asphyxial* stimulation, the nerves in the neck were not touched. The opening between the ribs, however, was much enlarged so as to permit a clear view of the nerves in the upper thorax. The typical arrangement of these nerves is shown diagrammatically in figure 1. On the right side the accelerator strands from the stellate ganglion join the vagus and pass down with it. On the left

Digitized by Google

side there is a trunk which passes directly toward the heart from the ganglion, and another which accompanies the vagus. It was our custom in operating for reflex and asphyxial stimulation to sever the vago-sympathetic trunk on the right, the vagus and the attendant trunk on the left, and also, very carefully, all fibers passing inward from the stellate ganglion on either side (at the points marked x, fig. 1) except that connecting the ganglion with the cervical sympathetic. Occasionally other nerve filaments were observed on the ventral side of the trachea near the heart; on the chance that these might be a source of

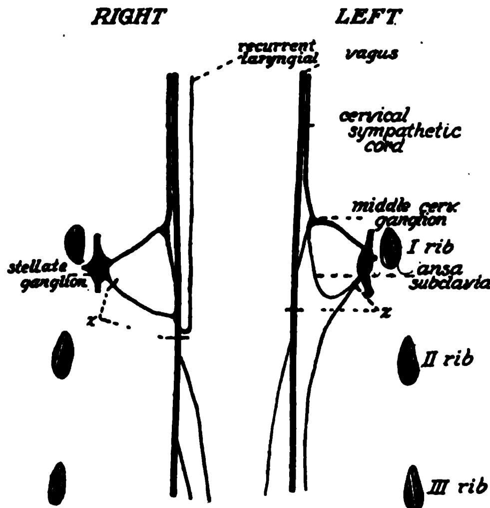


Fig. 1. Diagram showing the arrangement of the vagus nerves and the branches of the stellate ganglia in the lower neck and upper thorax of the cat. When the stellate ganglia were left in place the nerves were cut at the points marked x.

disturbance they also were cut. After thus doing all that was possible to isolate the heart without harm to the cervical sympathetic connections of the stellate ganglia, our standard of success was a fairly constant base line for the cardiac rate, and a return to that line after the rate had been disturbed by thyroid stimulation.

The effect produced by thyroid stimulation required often 3 hours and more for its completion. It is well known that the denervated heart is highly sensitive to alterations of temperature (7). Accordingly we were careful to maintain as nearly as possible a uniform temperature throughout the period of observation. The animal was laid on an

Digitized by Google

electric warming pad, the heat of which could be easily regulated. A sensitive thermometer in the rectum was examined every 10 minutes, and by properly adjusting the electric current the temperature of the animal was prevented from varying through more than a degree centigrade.

In the course of experimentation it soon became clear that positive results were seldom obtainable when the blood pressure was low, i.e., below about 70 mm. Hg. For example, on December 4, after a positive result had been obtained, and it had passed off, the blood pressure fell to 40 mm. Hg. when the adrenals were removed; thereupon a second attempt to obtain the result proved quite unsuccessful. On December 5, 8 and 9, after adrenalectomy, the pressure went down to about 50 mm. Hg., and the usual effect failed to appear. Again on December 22, after decerebration the pressure fell to 60 mm. Hg., and again there was failure. On January 2, with the pressure at 70 mm. Hg., the result was questionably positive. The negative results after adrenalectomy were not due to absence of the adrenal glands, as will be shown later. Since the heart is suffering from oxygen-want when the blood pressure falls below the critical level (8), it is probably unable to respond to the action of thyroid material. These failures should be compared with the successes which were obtained when the blood pressure was satisfactorily high.

To the difficulties with anesthesia, operation and low blood pressure was added an occasional difficulty with an initial high cardiac rate. In 20 cases the average rate of the denervated heart was about 200 beats per minute, with variations from about 180 to 220. These variations are in themselves of interest, but we have no explanation for them. Not very infrequently, however, the heart after being isolated from the nervous system would have a rate well over 240 beats per minute. Thus on January 6, an animal which had shown considerable excitement before and during anesthesia and which was actively digesting meat, had a pulse of 272 beats per minute, later falling to 240. On January 12, the rate after denervation of the heart (in a cat new to the laboratory) was 248, later 236. On January 29, the rate at the start was 252. When the heart was beating as rapidly as this we found that further attempts to accelerate it were futile. How to account for these occasional instances of very rapid pulse is not yet clear. It may be that the experience of the animal previous to anesthetization and operation was an important condition. For the present, however, we must leave the phenomenon unexplained. When such cases appeared, they were discarded as unserviceable.

Digitized by Google

The heart rate and blood pressure were registered by connecting with a mercury manometer the femoral artery (not the carotid because that would interfere with the circulation in the neck). Usually a record lasting 15 seconds was taken every 10 minutes during the period of observation.

The effect of thyroid massage. That a discharge of adrenin can be easily evoked by massage has been shown by several observers (9). It seemed possible that the thyroid also could be stimulated in the same way and would manifest a characteristic effect. Such stimulation has the advantage of being applied only to the gland that is being tested, and its effect limited to the region. The only other glandular structures which can be affected are the parathyroids, and if they are proved not to be influential, the result that occurs can only be attributed, mediately or immediately, to the action of the thyroid. This result can then serve as a standard by which to judge the results of exciting the thyroid by less direct ways.

That the rate of the denervated heart does not greatly vary if the animal, under uniform light ether anesthesia, is kept at a uniform temperature and its thyroid not stimulated, is shown in figures 2, 7 and 8. In the case represented in figure 2, the heart rates for an hour and a half before thyroid massage, registered every 10 minutes, varied only between 206 and 212 beats per minute. In that represented in figure 7 the variations for more than an hour lay between 196 and 204; and in that of figure 8 the range for an hour lay between 164 and 170.

In massage of the thyroid the gland was first laid bare and then stroked lengthwise with a smooth blunt dissector passed to and fro with slight pressure. This stroking was occasionally interrupted by gentle tapping. The massage rarely lasted more than 2 minutes. Never was it so vigorous as to cause any external bleeding or visible ecchymoses, though the gland might become slightly redder than before.

The effects of massage are shown in figures 2, 3, 4 and 5, and in table 1. As will be seen, the pulse is not much altered at first. Usually at the end of the 10-minute period following the stimulation, or in the next record thereafter, the rate is found to be accelerated. The acceleration rises to a maximum commonly in half or three-quarters of an hour; at this higher level the rate may remain for some time (in fig. 3, e.g., for about an hour). The increase has varied in our experiments from 16 to 50 beats per minute. After the maximum has been reached, and after the high level has been held for a considerable period, the rate gradually falls again to its former position. Two or 3 hours and more

Digitized by Google

may elapse before this restoration is complete (cf. figs. 3, 4 and 5 and also table 1). As shown in figure 3, massage of the other thyroid gland does not then reproduce the first effect. This may be merely

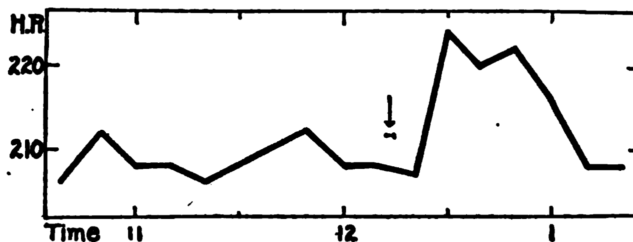


Fig 2. Graph showing absence of noteworthy variations of the heart rate during 100 minutes (10:40 to 12:20), and then a rise from 208 to 224 beats per minute, following massage of the right thyroid gland for 2 minutes (12:13–15).

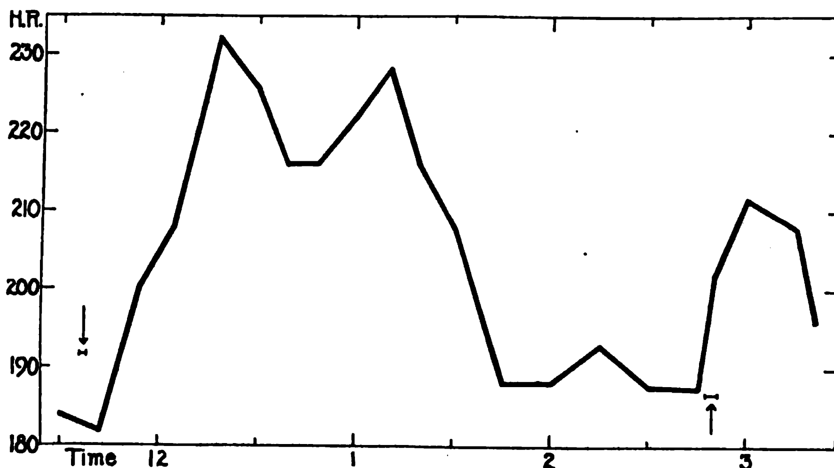


Fig. 3. Graph showing rise of heart rate from 182 to 232 beats per minute and persistent high rate for about 1 hour, induced by massage of the right thyroid gland for 2 minutes (11:36–38). Subsequent massage of the left thyroid for 3 minutes (2:47–50) caused a minor acceleration, from 188 to 212 beats per minute.

because the first effect has already occurred, but we have the impression that mere delay under ether and under the circumstances of operation renders the massage less capable of inducing a faster rate (cf. e.g., fig. 2).

Digitized by Google

That the faster rate induced by massage of the thyroid is not in fact due to disturbance of the parathyroids was proved by excising aseptically the one which is embedded on either side and later manipulating the thyroid alone. The effects were such as described above.

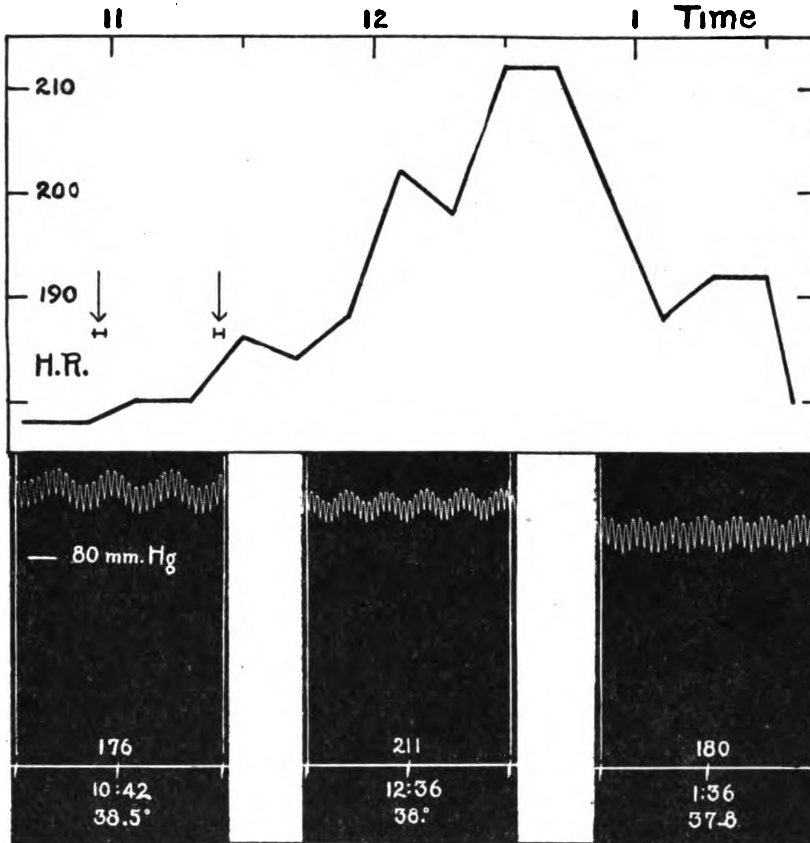


Fig. 4. Graph with samples of the original record showing absence of effect after massage of the right submaxillary gland (10:56–58), and increase of heart rate from 180 (11:18) to 211 beats per minute (12:36) after massage of both thyroid glands (11:23–25). The numbers above the line (zero blood pressure; 5-second intervals) here and in other similar records represent the heart rates per minute; the figures below, the time and the rectal temperature.

It might be supposed that other organs would share with the thyroid the capacity to accelerate the heart. Massage of the adrenal gland and also of the liver (5) can indeed have that effect, but it occurs at

Digitized by Google

once after the manipulation and passes away within a few minutes at the outside—i.e., before the *latent* period of the thyroid would be ended. In order to test another glandular structure, the submaxillary gland was vigorously stroked, pressed and tapped upon for 2 minutes. As shown in figure 4, no change resulted in the following 25 minutes. Then the thyroid was massaged in the usual way for 2 minutes. The cardiac rate soon began to rise and in about an hour had risen from 180 to 211 (31 beats) per minute. No greater amount of tissue disturbance, as such, was involved in manipulating the thyroid than in manipulating

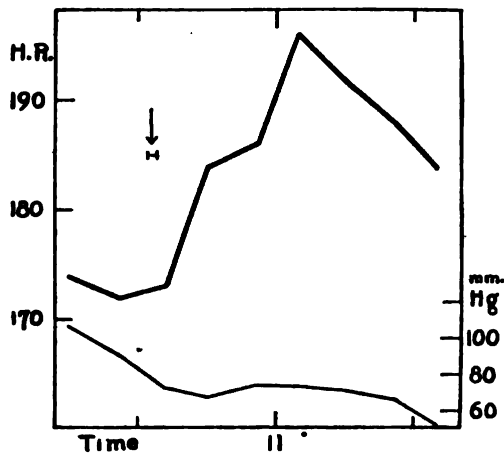


Fig. 5. Graph showing cardiac acceleration (from 172 to 196 beats per minute) after massage of the right thyroid gland for 2 minutes (10:32–34). The adrenal glands had been removed (at 9:35). As the blood pressure (lower line) fell below 70 mm. Hg., the experiment was interrupted.

the submaxillary, if, indeed, there was as much, and yet the effect was altogether different and characteristic when the thyroid was disturbed.

A question which early arose was whether the cardiac acceleration was due to the direct influence of thyroid substance carried to the heart, or was indirectly caused by thyroid stimulation of the adrenal medulla. As already remarked, the blood pressure was low in a number of experiments in which the adrenals were removed after denervation of the heart, and in these instances thyroid massage was ineffective. That this failure does not indicate that the adrenal glands are essential to the occurrence of a faster beat is shown in figure 5. Unfortunately about an hour after the stimulation the blood pressure fell below 70 mm.

Digitized by Google

Hg. and the experiment was therefore discontinued. The characteristic positive effect produced by thyroid massage in this and another instance, however, proves that the acceleration of the pulse is not mediated through adrenal secretion.

A single attempt to secure a faster heart rate by thyroid stimulation when the cardiac nerves were intact led to a questionable result. The

TABLE 1

Acceleration of the denervated heart by stimulation of the thyroid gland

DATE	HEART BEATS PER MINUTE			DURATION OF FASTER RATE
	Initial	Maximal	Increase	
Massage				
December 3	182	232	50	2 hours 40 minutes
December 4	208	224	16	40 minutes
December 6	124	150	26	3 hours
December 17	188	212	24	2 hours 10 minutes
January 10	204	220	16	50 minutes
February 4	180	211	31	2 hours 10 minutes
Cervical sympathetic stimulation				
December 13	192	220	28	3 hours 20 minutes
December 23	220	244	24	2 hours
January 5	144	172	28	3 hours 30 minutes
January 6	200	222	22	2 hours 10 minutes
Reflex stimulation				
January 13	176	208	32	3 hours +
January 22	212	240	28	2 hours
January 28	192	226	34	2 hours
Asphyxial stimulation				
January 31	198	216	18	1 hour 45 minutes
February 10	156	204	48	2 hours 30 minutes +
February 11	183	207	24	2 hours 20 minutes

heart rate which for 24 minutes had varied only between 190 and 192 beats per minute began to rise shortly after massage of the thyroid (12:42–45) and continued rising gradually until 1:42 when the rate was 212; there it remained for about 15 minutes, and then slowly returned to 200 beats per minute, at 2:30. The rise and fall were typical, and during most of the period the etherization was unchanged.

Digitized by Google

Before the thyroid stimulation, however, some vomiting movements (at 11:35) had sent the heart rate through the same range (192 to 212), with a return to 192 at 12:12. During this period the etherization was uniform. These incidental variations of rate when the nervous connections of the heart are intact render conclusions uncertain. We therefore relied on the denervated heart.

The effect of cervical sympathetic stimulation. In stimulating the cervical sympathetic we applied a mechanically interrupted tetanizing current to the strand in the neck in some instances and as it left the stellate ganglion in others. In different experiments the stimulation lasted from 5 to 15 minutes. The current was so weak that the iris was only about three-fourths withdrawn and was oscillating throughout the period as the stimulus went on and off.

The results are illustrated in figures 6 and 7 and in table 1. It will be seen that the heart is accelerated much as it is when the thyroid gland is directly massaged, and that the acceleration lasts for comparable periods.

It seemed possible that sympathetic impulses might affect other endocrine organs (e.g., the anterior lobe of the pituitary body) and that consequently the effect produced might be complicated by other than thyroid substance. In order to test this possibility we removed the thyroid on one side. We attempted to do this at the beginning of the experiment, but were confronted with a heart rate of 228 beats per minute, which in the course of 3 hours gradually fell to 180 beats. This might have been due to liberation of thyroid material during the removal of the gland. In order to avoid any possible thyroid effect except that in which we were interested, we removed the gland aseptically the day before the experiment. As shown in figure 7, stimulation (for 10 minutes—9:50–10:00) of the cervical sympathetic branch from the stellate on the side from which the thyroid had been removed had no effect. The nervous connections were intact, for the nictitating membrane was withdrawn and the iris was oscillating while the stimulus was intermittently applied. The same stimulation (for the same period—10:29–39) of the corresponding branch on the other side of the neck, where the thyroid was present, induced a typical increase of the heart rate. Within 10 minutes the pulse had begun to rise from the initial level of 200 beats and after 50 minutes it had slowly risen to 222. Thereupon the rate began to descend; in an hour and 10 minutes it had returned nearly to its initial level. The correspondence between the effects of thyroid massage and the effects of cervical sympathetic

Digitized by Google

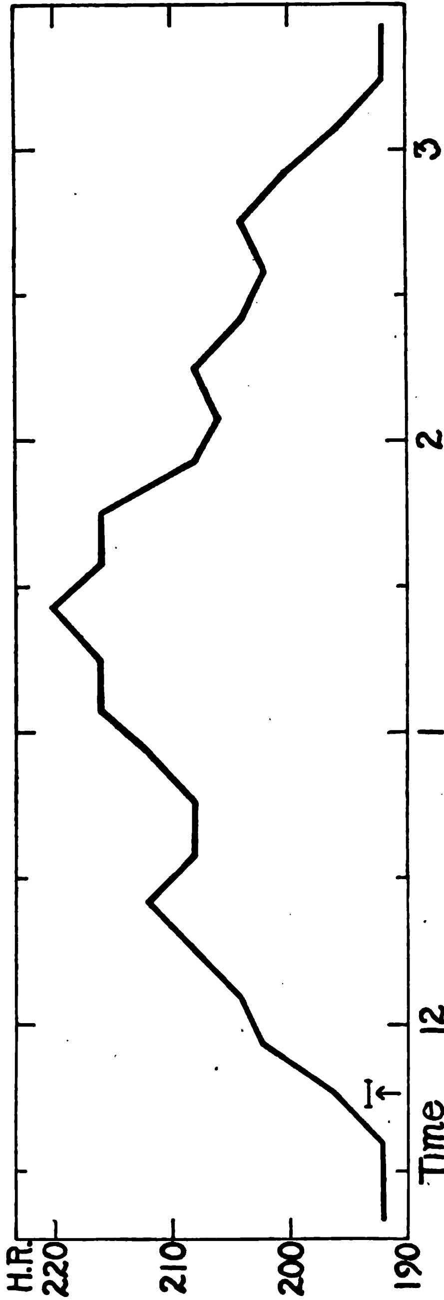


Fig. 6. Graph showing cardiac acceleration from 192 to 220 beats per minute, following stimulation of the right cervical sympathetic strand (11:43–48). The stimulus was a weak tetanizing current which caused a slight dilatation of the pupil and a slight retraction of the nictitating membrane.

Digitized by Google

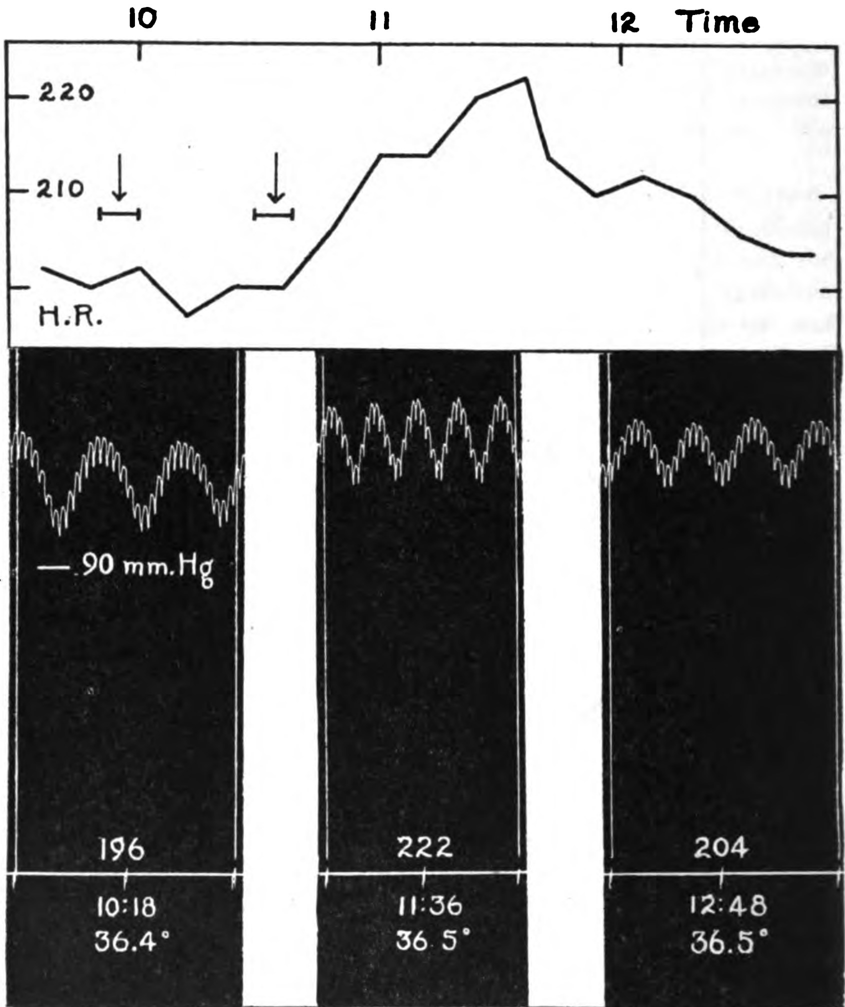


Fig. 7. Graph with samples of the original record showing absence of effect when the cervical sympathetic strand was stimulated (9:50–10:00) on the left side from which the thyroid gland had been removed, and a typical cardiac acceleration (from 200, at 10:24, to 222 beats per minute at 11:36) when the strand was stimulated (10:29–39) on the right side where the gland was present. The stimulus was in each instance a mechanically interrupted tetanizing current, just adequate to keep the iris oscillating.

Digitized by Google

stimulation when the thyroid is present (cf. figs. 4 and 7), and the absence of any influence of sympathetic stimulation when the thyroid is lacking seems to us to justify the conclusion that the faster cardiac rate is due to thyroid secretion induced by sympathetic impulses.

The effect of afferent stimulation and asphyxia. The rise of blood pressure which can be brought about by stimulation of a sensory nerve or by asphyxia is a manifestation of the efficacy of these measures in evoking sympathetic activity. Elsewhere evidence has been adduced

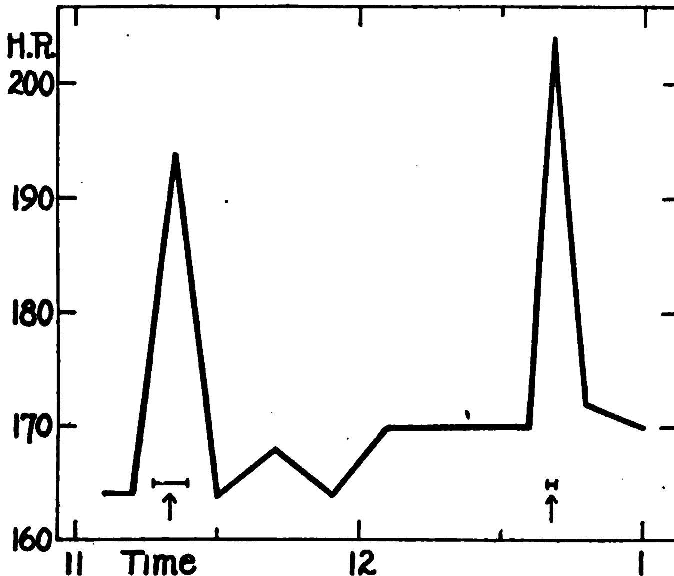


Fig. 8. Graph showing quick rise and fall of heart rate in consequence of afferent stimulation (sciatic nerve, 11:18–25) sufficient to cause slight dilatation of the pupil, and of asphyxia (12:40) for 105 seconds. *Both thyroid glands had been removed;* the accelerations are due to adrenal and probably also to hepatic secretion.

that these modes of exciting the sympathetic will provoke a secretion from the adrenal medulla, and possibly also from the liver, as revealed by a faster pulse (4), (5). The influence of these glands, therefore, would appear in any experiment demonstrating a reflex or asphyxial secretion from the thyroid. In order to have a background for judging the thyroid element in the response, as distinct from the adrenal and hepatic elements, we removed both thyroid glands aseptically and the next day recorded the change of rate of the denervated heart in response to afferent stimulation and asphyxia. The results are given in figure 8.

Digitized by Google

Interrupted tetanization of the sciatic nerve for 7 minutes (causing slight dilatation of the pupil) increased the pulse from 164 to 194 beats per minute. The heart promptly returned to its previous rate. There it remained during the following hour, varying only between 164 and 170. Asphyxia for 105 seconds then sent up the rate from 170 to 204 beats per minute; and again the heart promptly resumed its former pace, maintaining it thereafter without considerable change. It is not necessary now to discuss further the occasion for this cardiac accel-

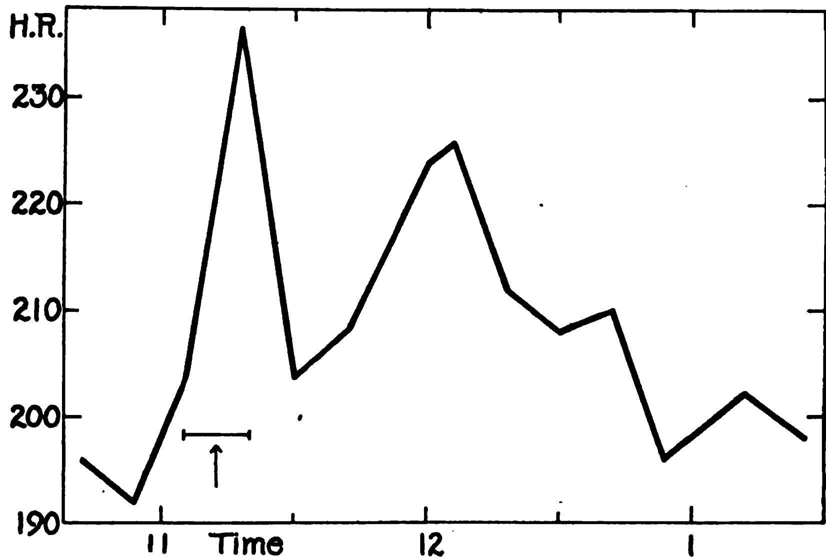


Fig. 9. Graph showing the quick rise and fall of rate shown in figure 8, followed by the slow rise and fall typical of thyroid stimulation. Both thyroid glands were present. The cardiac accelerations (from 192 to 236 and later to 226 beats per minute) were due to stimulation of the right brachial nerve with an interrupted tetanizing current from 11:05 to 11:20; only during the last 4 minutes were the pupils widened and the nictitating membranes withdrawn.

eration. As stated above, material discharged into the blood stream from the adrenal medulla and the liver accounts for the quick rise and fall of the heart rate in consequence of afferent stimulation and asphyxia. On the other hand, when these two modes of arousing sympathetic nerve impulses are applied when the thyroid glands are present, they give rise to characteristic additional changes of heart rate, resembling those already described as occurring after thyroid massage or after stimulation of the gland through the cervical sympathetic strand. These effects are shown in table 1 and also in figures 9 and 10.

Digitized by Google

In the experiment illustrated by figure 9 an interrupted tetanizing current (3 seconds on and 3 seconds off) was applied to the brachial nerve for 15 minutes. Only during the last 3 or 4 minutes was the anesthesia reduced to a degree that permitted reflex withdrawal of the

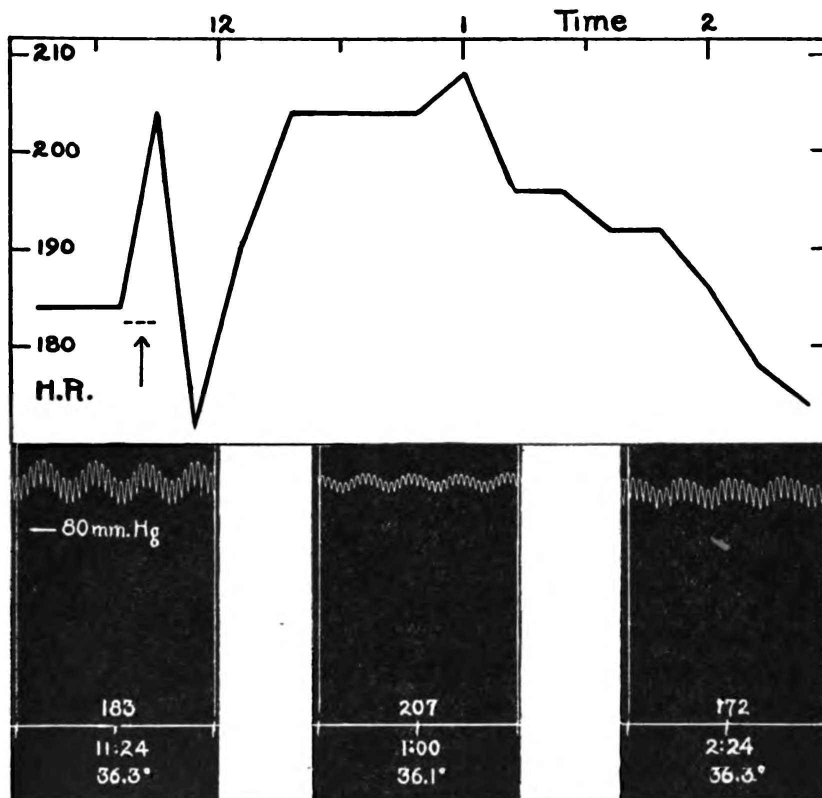


Fig. 10. Graph with samples of the original record showing the quick rise and fall of heart rate shown in figure 8, followed by the slow rise and fall typical of thyroid stimulation. Both thyroid glands were present. The cardiac accelerations (from 183 to 204 and from 172 to 207 beats per minute) were due to repeated periods of asphyxia, six in all, from 11:32 to 11:48, each lasting 1.5 or 2 minutes. The right nictitating membrane was completely withdrawn, the left nearly so, during the asphyxiation.

nictitating membranes and dilatation of the pupils. As the figure shows, the first effect was the typical sharp increase of rate (from 192 to 232 beats per minute) due to reflex adrenal (and hepatic?) secretion, and this was followed by the rapid fall shortly after the stimulation ceased.

Digitized by Google

Almost at once, however, the rate began to rise again and continued to rise in the slow manner characteristic of thyroid influence, until a maximum (226 beats per minute) was reached, and then slowly subsided to the former level. The absence of this second response when the thyroids are absent and its presence when they are present, together with the close resemblance of the positive effect to that produced by direct thyroid massage, justify the conclusion that it is due to reflex excitation of the thyroid glands.

Figure 10 illustrates the similar results of asphyxial stimulation. In this case the tracheal cannula was closed 1.5, 2, 2, 2 and 2 minutes in succession, with a period of breathing for 1 minute before each recurrent closure. The nictitating membranes were wholly withdrawn and the right pupil widely dilated (the left less so) during the period of repeated asphyxiation. Again there was a rapid rise of the cardiac rate from 183 to 204 beats per minute and an equally rapid fall when the stimulation ceased, a duplication of the first changes illustrated in figure 8 and properly ascribable to the discharge of adrenal and hepatic material into the blood stream. At once thereafter the rate began to rise and after a little more than an hour it had reached a maximum of 207 beats per minute. Another hour passed before the initial rate was restored.

In some of our cases the heart rate fell only slightly after the initial adrenal effect, i.e., the secondary thyroid influence very soon began to appear. On January 13, for example, the rate went up from 176 to 208 beats per minute during sciatic stimulation, and it had returned only to 192 when the fall was checked and the secondary rise began; it continued until a maximum (208 beats) equal to the adrenal effect was attained. Similarly in the asphyxial series (Feb. 10) asphyxia caused an acceleration of the heart from 156 to 232 beats per minute (an increase of 76 beats!). The rate dropped down to 194 and then slowly rose to 204 before starting on its gradual downward course.

General considerations. In contribution IV of this series Levy stated that though in his experiments adrenalin became more effective as a pressor agent after thyroid stimulation it did not become more effective as an accelerator of the heart. It is noteworthy, however, that in one case cited by him (2, p. 498) the basal rate of the heart increased 15 beats per minute after excitation of the left cervical sympathetic strand. This may have been an instance similar to those described in the foregoing pages, though the absence of a temperature record does not permit a definite conclusion to be drawn from his data. Certain it is that our present results confirm those of Levy and the earlier work of

Digitized by Google

Cannon and Cattell (1) in showing that the thyroid is capable of exerting a fairly prompt action and that the gland is subject to sympathetic control. The maximal heart rate in our experiments appeared sooner (in 30 to 45 minutes) after the stimulation than did Levy's maximal pressor effect of adrenalin (in 2 to 3 hours). This discrepancy may be related to a difference of action of thyroid material on the two structures —vessel wall and cardiac muscle—or it may be due to the very low blood pressure in Levy's pithed animals and the consequent retarding of all responses.

In an earlier paper of this series the results reported by Rahe, Rogers, Fawcett and Beebe (11) and by Watts (12), indicating that sympathetic stimulation reduces the iodine content of the thyroid on the side stimulated, were mentioned as supporting the view that the gland is subject to sympathetic impulses (1). Recently Van Dyke has repeated this work and has found that the variations in the iodine content on the two sides, stimulated and control, lie within the range of differences normally found in unstimulated animals (13). We know too little about either the character or the effective amount of the material given off by the gland to permit us to judge the significance of variations or the absence of variations in the iodine content. Even if Van Dyke's results are confirmed, the absence of chemical differences beyond those normally present in the two thyroid lobes could not be interpreted as contradicting the positive evidence of physiological effects of thyroid stimulation such as have been described in the foregoing pages.

In discussing sympathetic control of the gland Van Dyke mentions a number of investigators—Burget (14), Marine, Rogoff and Stewart (15), and Troell (16)—who have failed to obtain any effects on uniting the phrenic nerve with the cervical sympathetic strand, and he associates these failures with his own results as throwing doubt on sympathetic control of thyroid secretion. These failures, however, need not now concern us; they will be considered in a later paper when the positive experiments are described in detail; for the present we wish only to note that they have little or no bearing on the results we are now reporting.

In conclusion we wish to emphasize once more the importance of using the sympathetic fibers themselves rather than the superior or recurrent laryngeal nerves as means of testing the nervous government of the thyroid apparatus. Stimulation of the laryngeal nerves may induce effects such as we have obtained—indeed, we have seen acceleration of the denervated heart after exciting the superior laryngeal

Digitized by Google

branch of the vagus—but that does not prove that vagus impulses influence the gland. As pointed out in a previous paper (1), these *branches* of the vagus may have an admixture of sympathetic fibers and therefore the results of stimulating them are not decisive as to the division of the autonomic system that is effective. Our data in this and in previous communications show that it is the sympathetic division that acts—a fact of much importance in interpreting the function of the thyroid gland in the bodily economy.

SUMMARY

Gentle massage of the thyroid gland in the cat for 2 or 3 minutes will cause an increased rate of the denervated heart amounting in some instances to 25 per cent over the basal rate. The development of the maximal increase of rate is usually slow, requiring from 30 to 60 minutes and passing off in a similarly slow manner (see figs. 2, 3 and 4).

Massage of another gland, e.g., the submaxillary, does not cause this effect (see fig. 4).

The augmentation of heart rate caused by thyroid massage occurs in the absence of the adrenal glands (see fig. 5).

Stimulation of the cervical sympathetic trunk as it leaves the stellate ganglion induces a similar augmentation of the rate of the denervated heart; this does not occur if the thyroid gland has previously been removed (see figs. 6 and 7).

If the thyroid glands have been previously removed, sensory stimulation and asphyxia induce only the brief increase of rate due to adrenal and hepatic discharge (see fig. 8).

If the cardiac fibers from the stellate ganglia are severed, as well as the vagus nerves, and an afferent nerve, such as the sciatic or brachial, is stimulated under a degree of anesthesia which will permit reflex retraction of the nictitating membrane and dilatation of the pupil, there is a primary increase of rate due to adrenal secretion, followed by the slowly developing increase characteristic of the thyroid effect (see fig. 9).

If the vagi and the cardiac fibers of the stellate are cut, and the animal is asphyxiated under conditions which permit the eye changes described above, there is a similar primary rise due to adrenal secretion, followed by the secondary thyroid effect (see fig. 10).

Digitized by Google

Addendum. Attempts to repeat these observations in March and April have resulted in slight or negative effects. These rather striking differences are probably correlated with the remarkable seasonal variations in the iodine content of the thyroid gland reported by Seidell and Fenger (Journ. Biol. Chem., 1912, xiii, 523, Bulletin U. S. Hygienic Laboratory, No. 96, 1914, p. 67). These observers found that the percentage of iodine in the dried thyroid in March, though averaging one third, may be less than one eighth the percentage found in the late summer and autumn months.

BIBLIOGRAPHY

(1) Cannon and Cattell: This Journal, 1916, xli, 58.
(2) Levy: Ibid., 1916, xli, 492.
(3) Cannon and Rapport: Ibid., 1921, lviii, 308.
(4) Cannon: Ibid., 1919, l, 399.
(5) Cannon and Uridil: Ibid., 1921, lviii, 353.
(6) Evans: Journ. Physiol., 1912, xlv, 231.
(7) Knowlton and Starling: Ibid., 1912, xliv, 215.
(8) See Bayliss: Principles of general physiology, London, 1915, 680.
(9) See Stewart and Rogoff: Journ. Exper. Med., 1912, xxv, 547; Hoskins and McPeek: Journ. Amer. Med. Assoc., 1913, lx, 1777.
(10) Kendall: Collected papers, the Mayo Clinic, 1915, vii, 399.
(11) Rahe, Rogers, Fawcett and Beebe: This Journal, 1914, xxxiv, 72.
(12) Watts: Ibid., 1915, xxxviii, 356.
(13) Van Dyke: Ibid., 1921, lvi, 168.
(14) Burget: Ibid., 1917, xliv, 492.
(15) Marine, Rogoff and Stewart: Ibid., 1918, xlv, 268.
(16) Troell: Arch. Int. Med., 1916, xvii, 382.

Digitized by Google

THE EFFECT OF SOME POLYHYDRIC ALCOHOLS ON THE BEHAVIOR OF RATS IN THE CIRCULAR MAZE

DAVID I. MACHT AND GIU CHING TING

From the Pharmacological Laboratory, Johns Hopkins University

Received for publication January 18, 1922

In a preceding paper the authors have described their studies of the comparative effects of ethanol, caffeine and nicotine on the behavior of white rats in the circular maze (1). It was pointed out that this method of study is well adapted to the investigation of the narcotic properties of various drugs, as the maze enables one to determine even slight depression of the central nervous system and impairments of the neuro-muscular mechanism. Following the study of ethanol in this connection it was deemed desirable to inquire into the narcotic properties of small doses of other alcohols. It is well known that the higher mono-acid alcohols of the fatty acid series become more toxic with the increase in their molecular weight, following what is known as Richardson's law (2). A comparative study of primary and secondary alcohols carried out by one of the authors has also shown that while the secondary alcohols are less toxic than the primary ones, nevertheless the toxicity of analogous members increases as one goes up the series (3). It was therefore deemed useless to study the narcotic properties of the higher mono-acid alcohols. On the other hand the toxicity of some polyhydric alcohols is known to be not very great and a study was therefore undertaken to determine whether such alcohols are capable of depressing the brain and neuro-muscular mechanism of rats or, in other words, whether they exert a narcotic effect.

In the present investigation a number of such polyhydric alcohols was studied after injection intraperitoneally in white rats. The method of study was the same as described in the previous paper. Albino rats were trained to solve the maze problem so as to find their way through labrynthian paths to the center of the maze without committing any errors and in the shortest period of time possible. After the animals had been trained the various drugs to be studied were administered and the behavior as well as various somatic changes were noted at various intervals after the injection of the drugs.

Digitized by Google

Experimental data. A total of 40 rats was employed in this investigation. Most of the animals were male but a few experiments were also made on female rats. The animals at the beginning of the investigation were on the average about 45 days of age and in perfect physical condition.

The following polyhydric alcohols were studied: ethylene glycol $C_2H_4(OH)_2$, glycerol $C_3H_5(OH)_3$, erythrite, $C_4H_6(OH)_4$, arabite $C_5H_7(OH)_5$ mannite $C_6H_8(OH)_6$, dulcite $C_6H_8(OH)_6$, perseite $C_7H_9(OH)_7$ and volemite $C_7H_9(OH)_7$. Of these the first two are liquids while the others are crystalline solids. The drugs were dissolved in water, in concentrations of from 1 to 3½ per cent. Stronger solutions were not made in order to avoid mechanical irritation due to the salt action or the physical phenomenon of osmosis, etc. Control experiments were

TABLE 1

POLYHYDRIC ALCOHOLS	MINIMAL EFFECTIVE DOSE PER 100 GRAMS OF RAT
	mgm.
Ethanol C_2H_5OH	80
Glycol $C_2H_4(OH)_2$	120
Glycerol $C_3H_5(OH)_3$	160
Erythrite $C_4H_6(OH)_4$	290
Arabite $C_5H_7(OH)_5$	230
Mannite $C_6H_8(OH)_6$	320
Dulcite $C_6H_8(OH)_6$	120
Perseite $C_7H_9(OH)_7$	over 380
Volemite $C_7H_9(OH)_7$	over 380

made with injections of normal or physiological sodium chloride solutions, in order to determine the effect of injection of large volumes of fluid. It may be stated at once that injections of even 10 or 12 cc. of normal saline solutions produce very little or no effect on the rats half an hour after injection.

Results. The results obtained with the various polyhydric alcohols are expressed in the subjoined table. The object of the experiments was to determine the smallest quantity of the drugs used which produced a depression in the behavior of the rats. The average minimal effective doses in this respect are expressed in the table. It will be noted that glycol is quite depressant or, using the term in its broadest sense, "narcotic" for the rats, a dose of 120 mgm. per 100 grams of rat being sufficient to produce a depression in the behavior of the animals.

Digitized by Google

An excitement stage was not noted after any doses of the drug, as indicated by the method used.

The effect of glycerol or glycerine, the tri-acid alcohol, was also extremely interesting. The average minimal effective dose of glycerol was found to be about twice as great as that of ethyl alcohol, or 160 mgm. The higher members of the series were also found to produce depression provided a sufficient dose was administered. The tetra-hydric alcohol, erythrite, required 290 mgm. per 100 grams of weight to depress the animals. The pentra-hydric alcohol, arabite, required 230 mgm., while the two hexa-hydric alcohols studied, namely mannite and dulcite, required 320 mgm. and 120 mgm. respectively. It is interesting to note that the two isomers, mannite and dulcite, differed in their toxicity. The authors, however, did not pursue any further inquiries concerning the relative toxicity of various isomers of the various higher alcohols studied. Two members of the hepta-hydric alcohols were examined. These were the rare substances, perseite and volemite. These compounds, however, were found to be very little toxic, so that comparatively very large volumes of these solutions had to be employed and the results obtained while indicating a narcosis or depression were not entirely satisfactory as the depression might have been due to their salt action.

Discussion. It is evident from the above table that all of the substances examined produced a depression of the neuro-muscular system and are "narcotic" in the broad sense of the word. Of especial interest is the action of the first two members of the series; namely glycol and glycerol. Poison cases following the ingestion of glycol and glycerol are *not* unknown. Symptoms of intoxication in animals have been described by Dujardin, Baumetz and Audige (4) and others. References to intoxication following the ingestion of large quantities of glycerine have been recorded by Kobert (5) and Kunkel (6). In the present investigation. no excitement stage was noted after any of the drugs, even after small doses. It is interesting to note that while the potency of the drugs examined generally decreases with their molecular weight, exceptions occur as noted in the case of erythrite and arabite on the one hand and the isomers, mannite and dulcite, on the other. When one, however, compares the toxicity of the various drugs examined in their relation to their molar solutions the difference in toxicity is not so striking.

Digitized by Google

SUMMARY

1. A number of polyhydric alcohols was studied on rats in the circular maze.

2. It was found that all of the polyhydric alcohols when administered in sufficient quantity produced a depressant effect as indicated by this method.

BIBLIOGRAPHY

(1) Macht, Bloom and Ting: This Journal, 1921, lvi, 266.
(2) Richardson: Medical Times and Gazette, 1869, ii, 705.
(3) Macht: Journ. Pharm. Exper. Therap., 1920, xvi, 1.
(4) Dujardin, Baumetz and Audige: Recherches Exper. sur la puissance toxique des alcohols, Paris, 1879.
(5) Kobert: Lehrb. d. Intoxikationen, Stuttgart, 1906, ii, 666.
(6) Kunkel: Toxikologie, Jena, 1899, l, 427.

Digitized by Google

A COMPARISON OF WAVES OF BLOOD PRESSURE PRODUCED BY SLOW AND RAPID BREATHING

ROBERT T. TROTTER, PHILIPS EDSON AND ROBERT GESELL

From the Department of Physiology, University of California, Berkeley

Received for publication January 21, 1922

It has long been known that, synchronously with the phases of normal respiration, changes in blood pressure occur. In continuous blood pressure records these changes appear as waves. Each wave is completed during a single respiration, the rise and fall of pressure having a definite relation to inspiration and expiration (fig. 1). The object of this research was to compare these effects of respiration with changes in blood pressure produced by respiration of a rapid rate, using as a basis of study the observation that respiration, approximating closely the rate of heart beat, elicits long oscillations of blood pressure resembling those described above. These waves were observed by one of us in sacrifice experiments on the dog and cat in which for some unknown cause the respiration became markedly accelerated. Analysis of such waves shows that the series of heart beats associated with one complete oscillation of pressure is composed of one beat less (fig. 2) or one beat more (fig. 3) than the number of respirations occurring in the same interval of time. A rough analogy may be made between the cardio-respiratory waves of blood pressure produced under these conditions and the alternate reinforcement and interference of sound that occurs when two tuning forks of slightly different frequencies are made to vibrate simultaneously. We, therefore, refer to these oscillations as *cardio-respiratory interference* waves to distinguish them from what we designate as *simple cardio-respiratory* waves, in which the relation of respiration to heart rate is always that of one respiration to several heart beats.[1]

Knowing the nature of the cardio-respiratory interference waves, and the advantages (1) which the interference method offers for

[1] While the changes in pressure in the simple waves are in effect due to interference and augmentation by respiration, yet the interference is quite different from that referred to in the physical interference of sound. Bearing this in mind we feel that the names we have used will serve to differentiate the two main types.

Digitized by Google

investigation, the opportunity of producing these waves voluntarily in man was used to obtain a more extended series of blood pressure waves than the fortuitous experiments on the dog and cat would permit. The

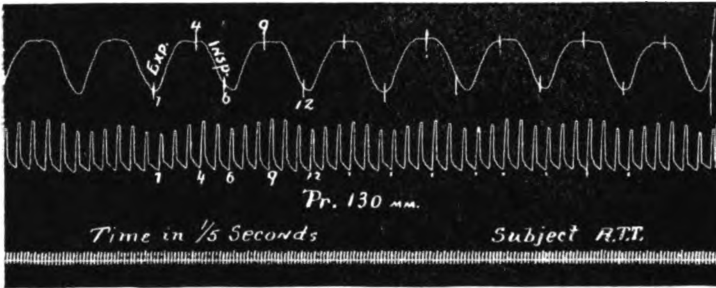


Fig. 1

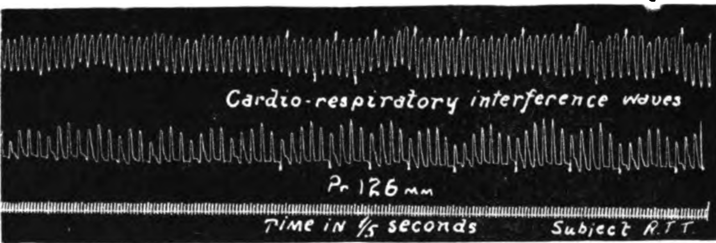


Fig. 2

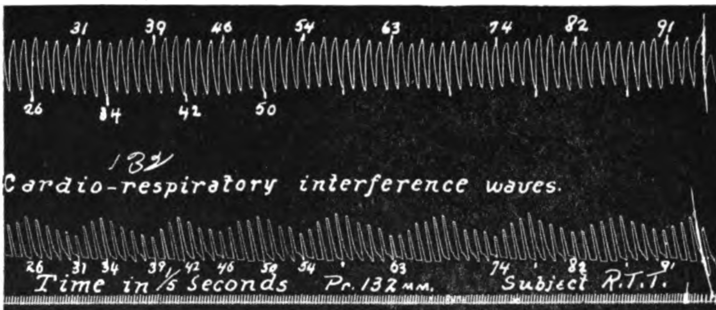


Fig. 3

experiments on man, therefore, supply the bulk of the data for this paper; and the experiments on the dog and cat are supplementary.

Method. Simultaneous tracings of blood pressure, respiratory movements, and time in seconds or fifths of seconds, were recorded upon

Digitized by Google

smoked paper by means of a long-roll kymographion revolving at a speed suitable for accurate measurements (figs. 4 and 5), the subjects being physiology students between the ages of 20 and 30 years, most of whom were men. Satisfactory records in each case comprised: *a*, simple cardio-respiratory waves; *b*, cardio-respiratory interference waves produced under two conditions—*1*, with respiration slightly faster than the heart rate, and *2*, with respiration slightly slower than the heart rate. Complete data which are summarized in figure 17 were obtained from thirty-two individuals.

Respiration was traced by means of a recording tambour suitably connected with a pneumograph made of a short metal cylinder, the ends being closed with rubber dam. The pneumograph was adjusted to the thorax in the region of the ensiform cartilage. There were a few exceptions to this procedure notably, in the case of women, where the adjustment of the pneumograph was higher up about the thorax. The subject was instructed to breathe synchronously with a metronome set to oscillate at the desired rate. For the simple cardio-respiratory waves the subject was instructed to breathe slowly and comfortably deeply, the metronome in most cases being dispensed with. No effort was made to have the respiration conform to any type such as "abdominal" or "thoracic."

Continuous blood pressure records were obtained by means of an Erlanger sphygmomanometer. The validity of the method has been demonstrated by Erlanger and Festerling (2) and their procedure for showing the effect of respiration on blood pressure has been largely followed in these experiments. The pressure in the cuff is maintained at a point slightly below systolic level[2] since it is at this pressure, according to Erlanger (2), that changes in the intra-arterial pressure give the greatest changes in the excursions of the writing lever. Under these conditions a rise in the intra-arterial pressure is indicated by an increase, and a fall by a decrease in the amplitude of oscillation.

[2] There seems to be considerable variation, in different subjects, of the optimum pressure for securing cardio-respiratory waves, particularly of the interference type. As the experiments proceeded somewhat lower pressures were used with better results. Figures 4 and 5 were taken in the neighborhood of diastolic pressure. All other records were taken much nearer the systolic than the diastolic level. Snyder (3) has shown that there is in most cases a reversal of the simple cardio-respiratory waves when the pressure in the cuff falls below the diastolic level. It is probable that with the pressure maintained in this region interference waves would suffer a similar reversal.

Digitized by Google

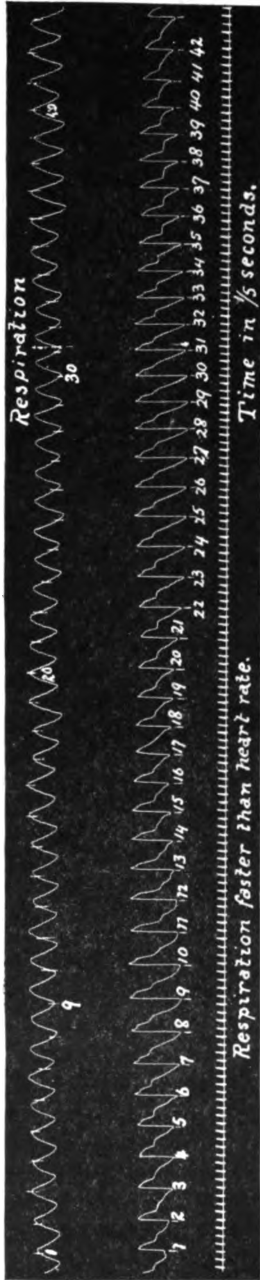


Fig. 4

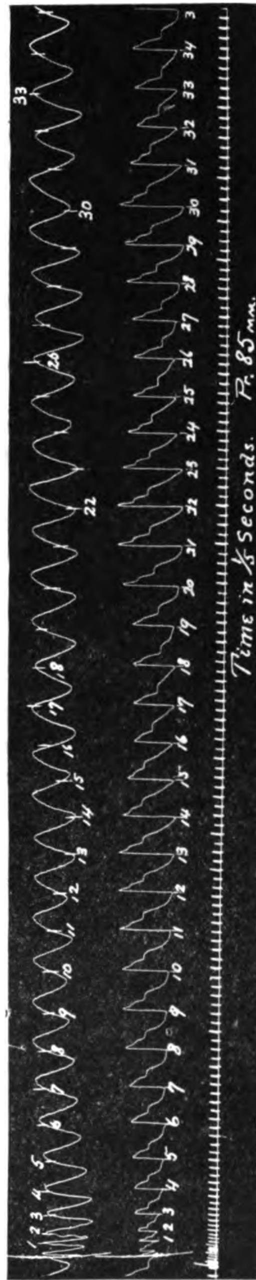


Fig. 5

Digitized by Google

In analyzing our records we have followed the common custom of laying off the heart beats on the respiratory tracing without making allowance for the time of transmission of the pulse to the cuff.

EXPERIMENTAL: *Simple cardio-respiratory waves of blood pressure.* All our records of blood pressure waves of this type (fig. 1) show, in the main, a rise of pressure during expiration, and a fall during inspiration (see fig. 17). This is in agreement with the findings of Erlanger and Festerling (2), but Snyder (3), in a record of twenty-eight cases, reports twenty as showing chiefly inspiratory rise and expiratory fall, and eight as showing chiefly expiratory rise and inspiratory fall of pressure.[3] In most of our experiments the pressure begins to rise toward the end of inspiration and to fall before expiration is completed (fig. 17). It is possible to get a complete reversal of the rise and fall in pressure by varying the depth and rapidity of breathing. We are in hearty agreement with Lewis (4, p. 254) when he says: "As respiratory curves of blood pressure are of very complex origin, and as the different factors involved in their production vary widely, it is not possible, either in man or animals, to state what the blood-pressure response to a particular respiratory act will be, unless the conditions and nature of the act are known."

Cardio-respiratory interference waves of blood pressure: When the rate of respiration is slightly faster or slightly slower than the heart rate we may conceive of cardio-respiratory cycles, each cycle comprising several heart beats and respirations, in which the number of respirations is greater by one, or less by one, than the number of heart beats. A cycle is completed when two beats (the first and last) fall at approximately the same time in respiration. When the heart rate is slower than the respiratory rate the pulsations of the heart fall progressively later in each respiration as they succeed each other. We shall henceforth speak of them as "advancing" along respiration. When the heart rate is faster than the respiratory rate the beats fall progressively earlier in each succeeding respiration, and we shall henceforth speak of them, under these circumstances, as "retreating" along respiration.

For the changes in blood pressure that occur in cardio-respiratory cycles we have already proposed the name of cardio-respiratory interference waves. It is convenient to think of each wave as beginning and ending in the pulsation of least amplitude. The length of the interference waves of blood pressure depends wholly upon how fast the

[3] For further discussion and bibliography on this subject refer to de Jager (5), Lewis (4), Erlanger and Festerling (2) and Wiggers (6), (7).

Digitized by Google

pulsations of the heart advance or retreat along respiration, it being evident that the faster the advance or retreat, the quicker the heart beats will complete a cycle, and the shorter the wave will be (see record in

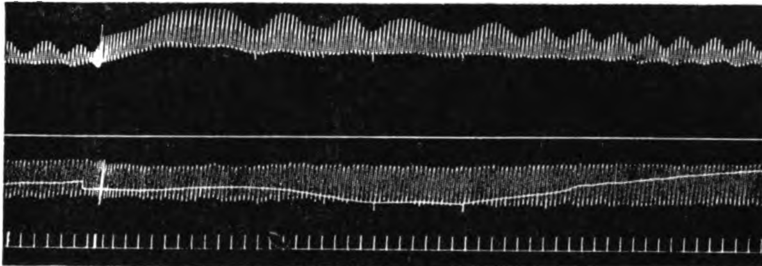


Fig. 6. Cardio-respiratory interference waves of the cat recorded with the Hürthle manometer connected with the carotid artery, showing a changing length of waves with a changing relative cardiac and respiratory rate. The upper tracing is the blood pressure, the middle is the respiration, and the lower the time record in seconds.

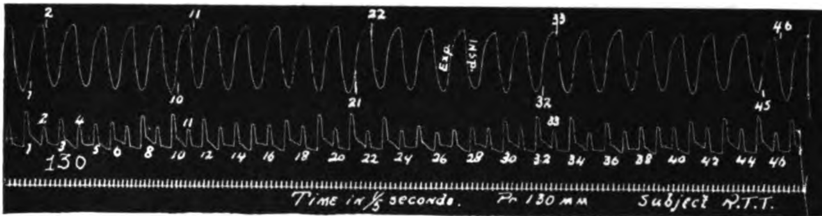


Fig. 7

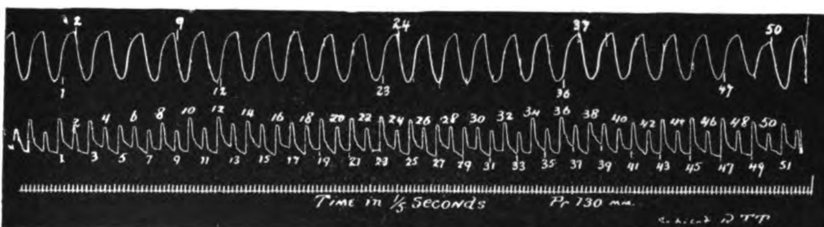


Fig. 8

fig. 6 which was taken from the cat, the blood pressure being recorded with the Hürthle manometer).

Cardio-respiratory interference waves may now be analyzed further according to whether they are produced by: *a*, respiration faster than

Digitized by Google

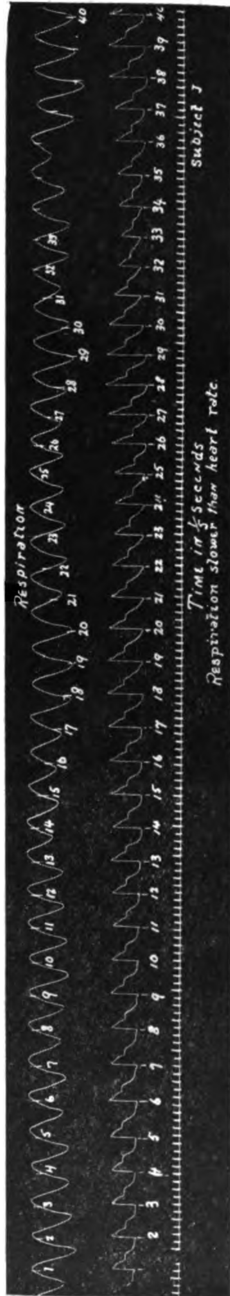


Fig. 9

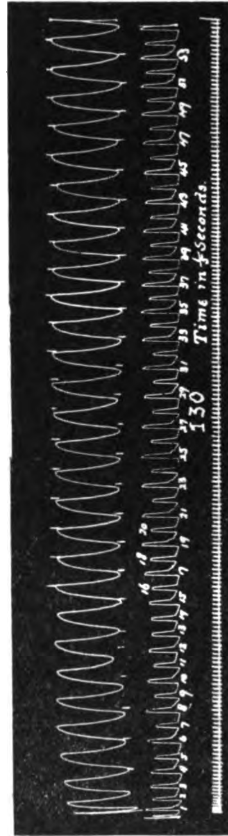


Fig. 10

the heart rate; *b*, respiration slower than the heart rate. For the sake of convenience, diagrammatic figures are used in the descriptions.

Figure 11a is a diagrammatic representation of a cardio-respiratory interference wave of blood pressure where the number of respirations is one more than the number of heart beats. The heart beats are laid off on the respiratory curve and numbered. It is apparent that the first four beats fall upon the inspiratory phases of respiration, while the remainder fall upon the expiratory phases. The relations of the blood pressure changes to respiration shown in figure 11a may be schematized in a simple form, shown in figure 11b, to make them more

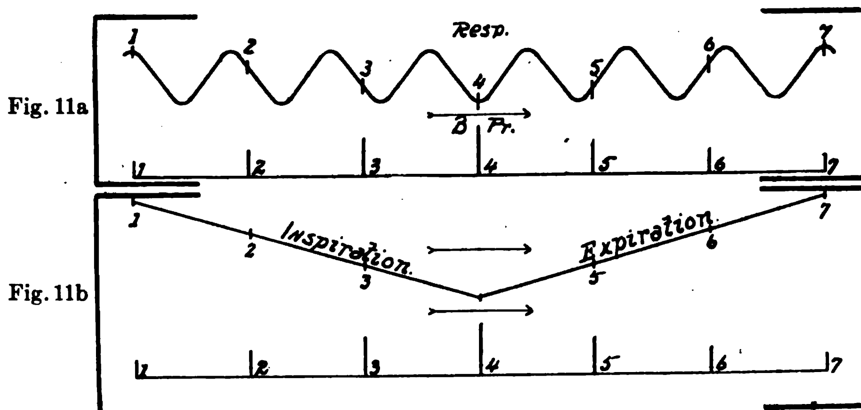


Fig. 11a is a diagram of a cardio-respiratory cycle, in which the respiration is faster than the heart rate. The pulsations advance along respiration, and are laid off on the respiratory curve. In figure 11b we have assembled all the beats on a single respiration in the same relative positions that they occupy in figure 11a. Note that the beats read from left to right, which is the direction of their movement along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively.

comparable to the changes occurring in the simple cardio-respiratory waves. Here we have taken a single respiration, respresented in the usual way (the downstroke being inspiration and the upstroke expiration), and have transferred to it all the heart beats in the cycle, placing them in their proper positions in inspiration and expiration. Since the heart rate is slower than the respiratory rate, the pulsations of the heart advance in respiration in the schema just as the pulsations advance in respiration in the simple cardio-respiratory waves. Both respiration and blood pressure tracings are read from left to right. The relations thus schematized may be interpreted in two ways: *a*, taking into considera-

Digitized by Google

tion the direction of the movement of the heart beats relative to the respiration we may say that, as the pulsations advance along inspiration there is a rise in blood pressure, as they advance along expiration there is a fall in blood pressure; *b*, in the second interpretation we omit entirely all consideration of relative motion, and consider only the position of the heart beats in the respiratory phases. Note that the beats of least amplitude fall at the beginning of inspiration, while the beats of greatest amplitude fall at the end of inspiration.

Figure 12a is a diagrammatic representation of a cardio-respiratory interference wave of blood pressure where the number of respirations

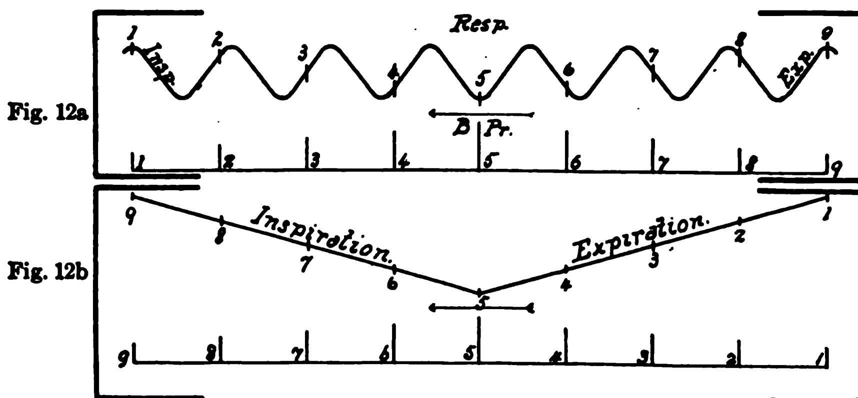


Fig. 12a is a diagram of a cardio-respiratory cycle, in which the respiration is slower than the heart rate. The pulsations retreat along respiration, and are laid off on the respiratory curve. In figure 12b all the beats are assembled on a single respiration in the same relative positions in which they fall in figure 12a. Note that the beats now read from right to left, which is the direction of their movement along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively.

is one less than the number of heart beats. The cardio-respiratory relations of figure 12a are schematically represented in figure 12b. Here again we have transferred to a single respiration all the heart beats in the cycle, placing them in their proper position in inspiration and expiration. The first five beats fall upon expiratory phases of respiration, while the last four fall upon inspiratory phases. Since the pulsations of the heart are retreating along, or lagging behind respiration (no. 1 falls at the end and no. 5 falls at the beginning of expiration), the blood pressure tracing in figure 12b must be read from right to left. The respiratory schema must, however, be read in the usual

Digitized by Google

way. The cardio-respiratory relations of figure 12b may likewise be interpreted in two ways: *a*, taking into consideration the direction of the movement of the heart beats relative to respiration we may say that, as the pulsations retreat along expiration there is a rise in blood pressure (it should be pointed out, however, that such a sequence of respiration and heart beat cannot possibly obtain in the simple cardio-respiratory wave); *b*, in the second interpretation disregarding entirely consideration of relative motion, and considering only the relative positions of the heart beat and respiration, we note that the beats of least amplitude again fall at the beginning of inspiration, while the beats of greatest amplitude fall at the end of inspiration. The waves differ only when the direction of the movement of the heart beat on the respiration is considered.

In figure 17 we have summarized graphically some of the data obtained from thirty-six subjects. The interference waves are plotted with reference to respiration according to the manner already described. The simple waves are plotted as they occur in the records. Each wave must be referred to the respiratory curve at the top of the graph. In no case is the amplitude of oscillation recorded. We have contented ourselves with recording the points of lowest and highest pressures, the lines connecting these points merely show that the pressure rises or falls as the case may be in the interval. It is evident that we have plotted a single wave only of each type. We have, however, analyzed many others, and have found almost invariably the same cardio-respiratory relations. We have in our records a few instances of simple and interference waves in which the cardio-respiratory relations are the reverse of what we have depicted, but these were always the exceptions even in the records in which they occurred. The numbers indicate the length of the individual waves as well as the number of beats making up the rise or the fall in pressure. Observe that the pressure changes of the interference waves follow more closely the changes of respiratory phase than do the simple waves.

Some very interesting effects may be obtained by having the subject breathe slightly slower, or slightly faster than half the heart rate. Under these circumstances we may get two sets of cardio-respiratory waves in progress simultaneously. One set is composed of the even-numbered, and the other of the odd-numbered beats. When one wave of blood pressure is at its crest the other is at its trough.

Figure 13a is a diagrammatic representation of a record such as figure 7, in which respiration is slightly quicker than one-half the heart

Digitized by Google

rate. The most obvious characteristic of such a record is an apparent alternating beat. Further analysis, however, shows that there are two sets of interference waves in progress at the same time, one being composed of the even-numbered, and the other of the odd-numbered beats. Figure 13b shows these waves plotted separately in their proper

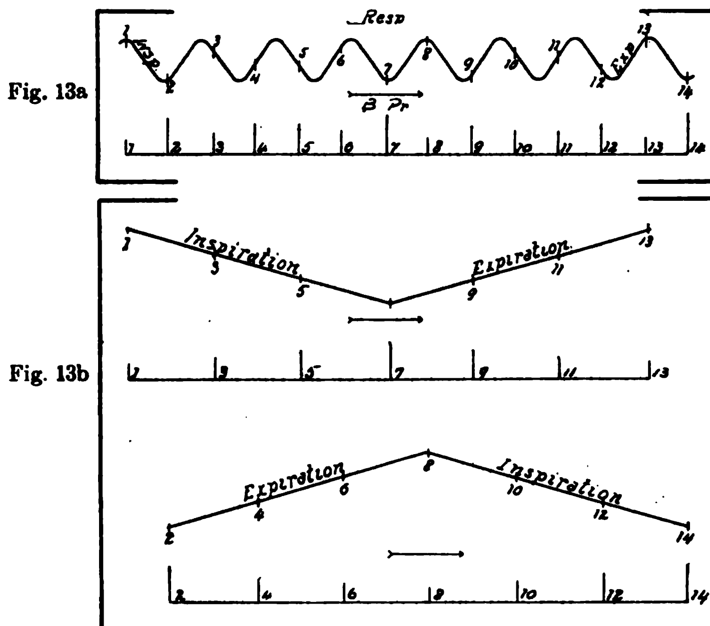


Fig. 13a is a diagram of double cardio-respiratory interference waves. The respiratory rate is slightly faster than half the heart rate. The pulsations advance along respiration, and are laid off on the respiratory curve. In figure 13b the odd and even-numbered beats respectively are assembled on single respirations in the same relative positions that they occupy in figure 13a. Note that the beats read from left to right, which is the direction of their movement along respiration. The points of highest and lowest pressures correspond to the beginning and end of inspiration respectively. Each of the double interference waves is identical with the type shown in figure 11b.

relation to respiration. Comparison of the two waves shows that they are of the same type, and that they bear identical relations to respiration. They are furthermore identical with the type of interference wave shown in figure 11b.

Figure 14a is a diagrammatic representation of a record such as figure 8, in which respiration is slightly slower than one-half the heart rate.

Digitized by Google

The double interference waves thus obtained are separated and shown schematically in figure 14b in their proper relation to respiration. Compare these waves with each other and with the interference wave represented in figure 12b. They will be seen to have identical relations.

The cardio-respiratory interference waves, whether of the usual (single) or alternating beat (double) types, have upon analysis shown

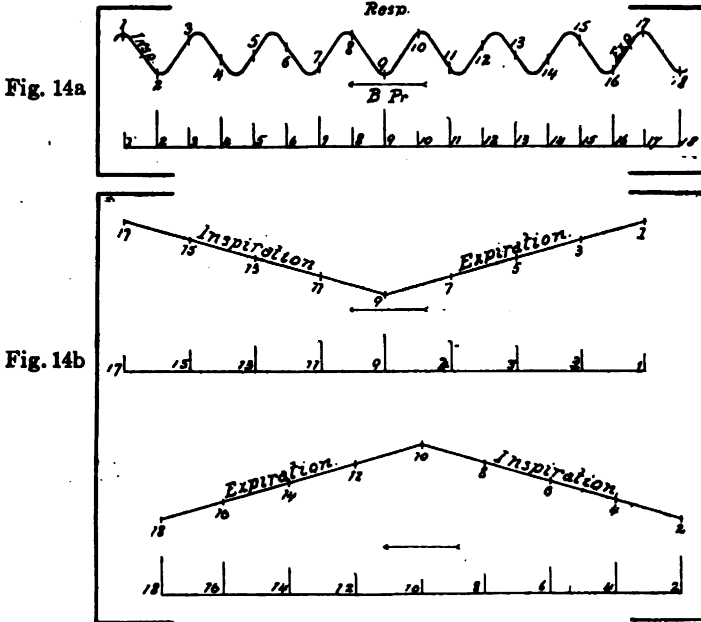


Fig. 14a is a diagram of double cardio-respiratory interference waves. The respiratory rate is slightly slower than half the heart rate. The pulsations retreat along respiration, and are laid off on the respiratory curve. In figure 14b the odd and even-numbered beats respectively are assembled on single respirations in the same relative positions that they occupy in figure 14a. Note that the beats now read from right to left, which is the direction of their movement along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively. Each of the double interference waves is identical with the type shown in figure 12b.

a relation to both phases of respiration, but records have been obtained which show that waves of blood pressure may be obtained that are related to either inspiration or to expiration alone.

Figure 15 is a diagrammatic representation of a wave of blood pressure that is related to inspiration only. From the beginning of inspiration

Digitized by Google

the beats advance till they reach the end of inspiration. At this point there is a change in the heart rate which causes them to retreat along the same respiratory phase. Figure 16 shows a wave related only to expiration. There is first a retreat and then an advance along this phase. These waves of blood pressure are equivalent to true inter-

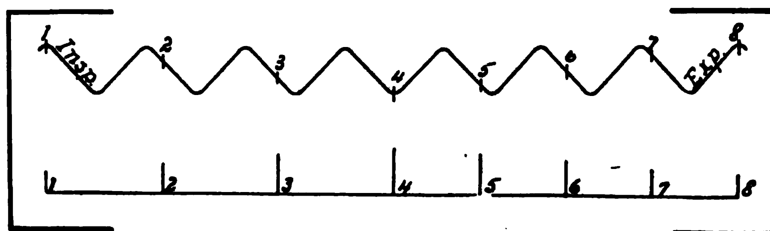


Fig. 15 is a diagram of a cardio-respiratory interference wave that is a combination of the two types of interference waves shown in figures 11a and 12a. Note that all the beats of the wave fall on inspiratory phases. From 1–4 the heart rate is slower than respiration, while from 4–8 the heart rate is faster than the respiratory rate. Consequently the heart beats first advance and then retreat along respiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively. In the record shown in figure 4 there is a wave of this type that is made up of beats 20–40.

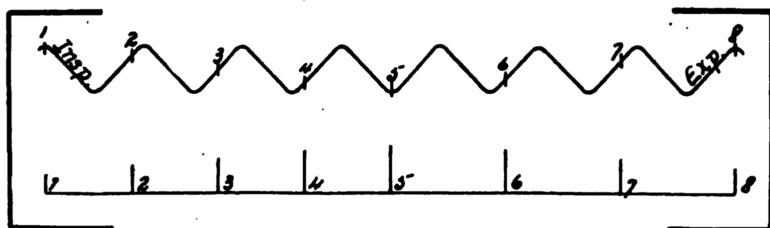


Fig. 16 is a diagram of a cardio-respiratory wave that is a combination of the two types of interference waves shown in figures 11a and 12a, but in this case all the beats of the wave fall on expiratory phases. From 1–5 the heart rate is faster than respiration, while from 5–8 the heart rate is slower than the respiratory rate. Consequently the heart beats first retreat and then advance along expiration. The points of lowest and highest pressures correspond to the beginning and end of inspiration respectively.

ference waves in spite of the fact that in the cardio-respiratory cycle the number of respirations is equal to the number of heart beats. Each half of a wave of this type has a relation to respiration that conforms to the conditions under which interference waves were found to occur. The changes in the heart rate that give rise to these waves are obviously

Digitized by Google

opportune, yet we have noticed several cases in our records where they occur.[4]

Up to this point we have dealt only with a shifting time relation between the pulse and the respiratory rates associated with changes in blood pressure, which appear to be related to respiration. Figures 9 and 10 show what occurs if the respiratory rate equals exactly *a*, the heart rate, or, *b*, one-half the heart rate. These conditions, difficult to obtain deliberately, are sometimes realized fortuitously. In figure 9 the heart and respiratory rates are very nearly the same from pulsations 5 to 12, and consequently the heart beats fall in approximately the same relative positions on respiration. As long as this relation is maintained there is no change in the amplitude of the pulse beats. Beyond pulsation 12, however, each successive beat falls earlier in respiration. The effect on the blood pressure is immediate; cardio-respiratory interference waves begin to form. In figure 10 the respiratory rate is for the greater part exactly equal to one-half the heart rate. Here we see the same phenomenon illustrated, namely, that beats which fall in the same relative position on different respirations do not change in amplitude.[5] Again we find that the beats of least amplitude fall roughly at the beginning of inspiration, while those of greatest amplitude fall roughly at the beginning of expiration.

Records such as figures 7, 8 and 10, occurring in the normal individual, suggest a possible new explanation for some cases of pulsus alternans.[6]

[4] It is obvious that a suitable change in the respiratory rate at the critical moment would be as effective in producing these waves as a change in the heart rate. Since respiration is under voluntary control, one should be able to secure them at will from a good subject.

[5] Figure 10 may be interpreted in other ways. This record may be considered as an example of the shortest possible interference waves. The cardio-respiratory cycle being made up of one respiration to two heart beats conforms to the conditions under which we have found these waves to occur (there is an excess of one heart beat over the number of respirations in the cycle). On the other hand, since the blood pressure changes are complete in the period of a single respiration, we might equally well call figure 10 a record of simple cardio-respiratory waves. Evidently the simple and interference types of waves are merged into one.

[6] As this paper is completed for the press our attention is called to a review (Journ. Amer. Med. Assoc., Oct. 22, 1921) of a paper by Aguilar on "Respiratory False Pulsus Alternans," occurring in a man suffering from bradycardia of nodal origin. It would be equally interesting to determine whether or not pulsus alternans of respiratory origin occurs in individuals suffering from dyspnea associated with rapid respiration.

Digitized by Google

Results on the dog and cat. The infrequency of distinct cardio-respiratory interference waves in the dog and cat, occurring as they do by accident, hardly warranted a series of experiments for their study alone. We have, however, collected some data in the course of other experiments which supplement the results on man. In these experiments (two on the dog and one on the cat) respiration was recorded as in man: blood pressure in the dog with the mercury manometer, in the cat with the Hürthle manometer. Cardio-respiratory interference waves with the respiratory rate approximately equal to the heart rate, and also to one-half the heart rate, were obtained. During the course of an interference wave in the dog, the mean blood pressure was highest when the pulse occurred at the end of inspiration, and lowest when the pulse occurred at the beginning of inspiration. In the cat both systolic and diastolic pressures showed the same relations to respiration; systolic pressure, however, showed the greatest fluctuations. Using pulse pressure as an index, cardiac output was greatest at the end of inspiration. In one experiment, in which simple cardio-respiratory waves were obtained as well as interference waves, the changes in blood pressure were similar to those reported in man. Therefore, the data obtained in the three experiments on the dog and cat are in agreement with the results reported above.

DISCUSSION

If figures 2, 3, 4, 5, 6, 7 and 8 are compared it will be evident that all the records show in the main the same thing, namely, that the pul-

---

Fig. 17 is a graphic summary of a single blood pressure wave of each type obtained from the different subjects, the data being complete for thirty-two individuals.

Column 1 summarizes the data on simple cardio-respiratory waves of blood pressure.

Column 2 shows the cardio-respiratory relations of the interference waves where the respirations are in excess over the heart beats by one, that is, respiration is faster than the heart rate.

Column 3 shows the respiratory relations of interference waves where the heart beats are in excess over the respirations by one, that is, respiration is slower than the heart rate.

Corrections are marked for the onset of auricular and ventricular systole, indicating the distance each wave of blood pressure must be shifted to the left to show the relation of auricular and ventricular systole to the respiratory cycle. These corrections are obviously only approximately correct, and apply only to the waves of the interference type (columns 2 and 3). The corrections for auricular and ventricular systole in the simple waves are negligible owing to the greater average duration of each respiration.

Digitized by Google

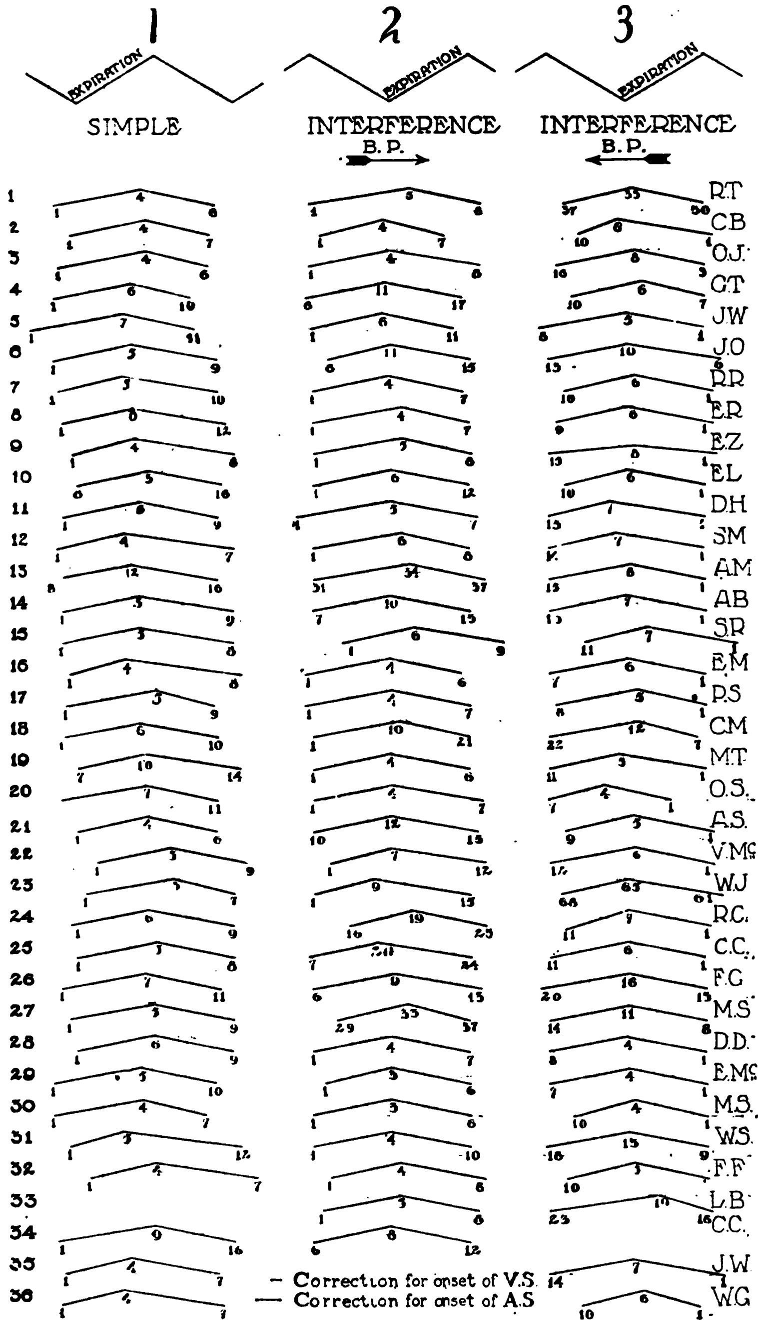


Fig. 17

sations of least amplitude fall close to the beginning of inspiration (or end of expiration), while the pulsations of greatest amplitude correspond more or less closely to the end of inspiration (or beginning of expiration). Consequently the pressure rises as the heart beats approach the beginning of expiration, irrespective of the direction of the movement of the pulse beats along respiration. Similarly the pressure falls as the heart beats approach the beginning of inspiration, whether the approach is made from an advance along the expiratory phase or from a recession along inspiration. In other words, every phase of inspiration and expiration has its own quantitative effect upon blood pressure. The double interference waves will be easily understood if these points are kept in mind.

A comparison of our simple cardio-respiratory waves with the interference waves shows that the points of lowest and highest pressures are reversed in the two kinds of waves (see fig. 17). In the simple waves, although the correspondence is in many cases far from exact, we have found almost invariably an expiratory rise and an inspiratory fall of pressure. Hence the point of lowest pressure corresponds roughly to the end of inspiration, and the point of highest pressure to the beginning of inspiration. In the interference waves, on the other hand, regardless of the conditions under which they are produced, the points of lowest and highest pressures on the respiratory tracing correspond roughly to the beginning and end of inspiration respectively. An attempt to explain these differences would involve us in an analysis of the nature and action of the forces that produce the simple waves of blood pressure. Since we have made no contribution to the subject, and since there is so little agreement in the literature, we do not feel justified in doing more than compare their respiratory relations, as we found them, with those of the interference waves.

Neither is it within the scope of this paper to attempt a final explanation of the cause of interference waves, yet we would like to call attention to some considerations that may have a bearing on the question. The quick changes of blood pressure seen in the double (alternating beat) interference waves are probably too rapid to be explained by varying pulmonary blood capacity, or changing pulmonary resistance; and further, in no type of interference waves are the respiratory changes in heart rate comparable to those occurring in the simple waves.[7]

[7] One subject (S.M.) did indeed give clear indications of cardiac variations that appeared referable to respiration. These were, however, transitory, and many of the waves showed no such changes. The question is one that needs further investigation.

Digitized by Google

For these reasons we believe the production of interference waves to be due primarily to rapid changes in the movement of blood in and about the base of the heart, resulting from changing intra-thoracic pressure.[8]

SUMMARY

The effects of rapid breathing were compared with those of more normal breathing upon the systolic blood pressure in man. Supplementary data were also obtained on the dog and cat.

For the well-known changes of blood pressure that occur during a single respiration, and which are more or less synchronous with the changing respiratory phases, we have proposed the name of *simple cardio-respiratory waves* to distinguish them from those waves produced by rapid breathing.

The oscillations of pressure elicited during rapid breathing by the interference method we have designated as *cardio-respiratory interference waves.*

The most striking difference in the respiratory relations of the simple and interference waves is that in the simple waves the blood pressure changes are complete within the period of a *single* respiration, while in the interference waves the gamut of the blood pressure changes is run through in the interval of *several* respirations.

[8] We have already called attention to the fact that in analyzing our records we have made no correction for the time necessary for the transmission of the pulse to the cuff. Neither have we made correction for the beginning of ventricular or auricular systole, but have determined the position of the heart beats with reference to the appearance of the pulse at the cuff to make the results comparable with those reported in simple cardio-respiratory waves. These results are summarized in figure 17. We have added two corrections to this figure, showing the moment of onset of ventricular and auricular systole in the respiratory cycle corresponding to the pressures which are plotted. For example, to determine the relation of the onset of ventricular systole associated with the highest pressure to respiration, the blood pressure wave should be shifted to the left, the designated distance equivalent to 0.06 second. To determine the same relation for the onset of auricular systole, the curve should be shifted in the same direction, the designated distance corresponding to 0.16 second. In making these corrections the points of high and low pressures will now be found to anticipate the respiratory changes of phase. If we accept the common view that an increase in the negative pressure of the thorax favors venous filling by its aspiratory action, we might expect the points of highest pressure to correspond with the onset of auricular systole and the end of inspiration. But we cannot say with certainty just how changing intra-thoracic pressure acts. The difference in thickness of the left auricle and the left ventricle might, for example, hinder the flow of blood from the auricles to the ventricles during auricular diastole when inspiration is in progress.

Digitized by Google

The production of interference waves of blood pressure is dependent upon the establishment of cardio-respiratory cycles, in which the number of respirations is greater by one or less by one than the number of heart beats making up the waves and occurring in the same time interval.

When these conditions are fulfilled we may conceive of the heart beats as moving through respiration, the direction of the movement being determined by the relative rates of the heart and respiration; that is, whether the respiratory rate is slower or faster than the heart rate. A cardio-respiratory cycle is complete when two beats (the first and last of the interference wave) fall at approximately the same time in respiration.

We have found that, whereas in the cardio-respiratory interference waves the highest and lowest pressures were associated approximately with the beginning of expiration and of inspiration respectively, in the simple respiratory waves these relations were reversed; that is, the points of highest and lowest pressures correspond roughly to the beginning of inspiration and of expiration respectively.

Without definitely assigning the responsibility for the production of interference waves to any particular respiratory factor, we are inclined to favor the hypothesis that they are primarily due to the changing intra-thoracic pressure accompanying respiration.

It is possible, by breathing slightly slower or slightly faster than half the heart rate, to produce double interference waves of blood pressure; that is, under these conditions, two waves of blood pressure may be in progress simultaneously. Each of the double waves is formed by alternate heart beats, one being made up of the even-numbered and the other of the odd-numbered beats. Double cardio-respiratory interference waves are to be explained in the same manner as the single waves.

Supplementary data obtained from experiments on the dog and cat are in agreement with those obtained in man.

Cardio-respiratory interference waves particularly of the double type with alternating beats occur spontaneously in sacrifice experiments in dogs and cats. We, therefore, point to our work as occasionally explaining pulsus alternans and blood pressure waves of the third order occurring in man.

BIBLIOGRAPHY

(1) Gesell: This Journal, 1916, xl, 267.
(2) Erlanger and Festerling: Journ. Exper. Med., 1912, xv, 370.
(3) Snyder: This Journal, 1914, xxxvi, 430.
(4) Lewis: Journ. Physiology., 1908, xxxvii, 233.
(5) de Jager: Journ. Physiol., 1886, vii, 130.
(6) Wiggers: Circulation in health and disease, 1915, 95.
(7) Wiggers: Physiol. Rev., 1921, i, 250.

Digitized by Google

STUDIES ON ALKALIGENESIS IN TISSUES

I. Ammonia Production in the Nerve Fiber during Excitation[1]

SHIRO TASHIRO

From the Department of Biochemistry, University of Cincinnati

Received for publication January 28, 1922

Immediately after the publication of an article (1) in which the author reported that the resting nerve respires and that this respiration increases during the passage of the impulse, he made some attempts to devise a quick and easy method practicable for ordinary class experiments to demonstrate this increased metabolism during the stimulation. On account of the necessity of an elaborate device to make air free from CO_2, the principle used in his original apparatus could not be used. Since we know today more about the proper use of indicators than in early days, when physiologists used them for detection of CO_2 in metabolism experiments on nerves without any degree of success, and since we know the exact amounts of CO_2 produced in the nerve under various conditions, the indicator method was next tried. We immersed the nerve in Ringer's solution, saturated with phenolphthalein and containing enough alkali to give a slight pink color but not enough to affect appreciably nervous activity. Although this method was good enough to show that the resting nerve gives off an acid (CO_2), yet it would not show a decided difference in rates of decolorization in tubes containing resting and stimulated nerves.

This failure of detection of an increased carbon dioxide by the stimulated nerve meant one of two things. Either the $Ba(OH)_2$ method the author previously used was wrong and the stimulated nerve does not give any more CO_2 than the resting, or the increased CO_2 production is masked when an indicator method was used. Because of several hundred repetitions with the same results of his original method, demonstrated to many and confirmed by all of his students who learned the proper use of his apparatus, this apparent failure with the indicator

[1] Preliminary report of this work was given before the Chicago Meeting of the American Physiological Society, 1920, and its abstract appeared in this Journal, 1921, lv, 282.

Digitized by Google

method was not taken by him as conclusive evidence against his original contention but was considered to be due to some difficulty with the indicator method.

With an assumption, therefore, that his original method of estimating CO_2 is accurate and that CO_2 production does increase in the nerve during excitation, we proceeded to inquire what could be the interfering factors if the indicator method was used.

There are two obvious conditions which might mask the increase of acidity due to production of more CO_2—one is the simultaneous production of any base-forming compounds; the other is the presence of buffers in the tissue itself or its production in or diffusion out of the tissue.

During preliminary experiments, the author discovered that when two nerves of equal weight were placed in chamber A and B of his CO_2 apparatus and hemispheres of Nessler's solution were introduced instead of $Ba(OH)_2$, the Nessler drop in the chamber in which the stimulated nerve is placed gave a brownish precipitate much quicker than that of the resting nerve. This suggested the possibility of more NH_3 formation in the stimulated nerve, but it was not necessarily NH_3 since CO_2 is known to give a precipitate with Nessler. To scrutinize this difficulty, drops of NaOH solution were placed in both chambers for the purpose of eliminating any CO_2 which we knew to be produced. The same result could be obtained as before, showing that the precipitate was not due to CO_2, but it was impossible to use this method for estimating quantities. The first difficulty was in producing a perfectly clear Nessler drop which is the essential requirement for quantitative determinations in the biometer. The second difficulty was that the stopcock caused a great deal of trouble on account of its strong alkali. The substitution of a pinchcock for the stopcock caused a further difficulty in that it was impossible to place a stationary hemispherical drop of Nessler's reagent on the top of the tube.

In spite of these difficulties and the lack of quantitative data, however, the conclusion was warranted that there is at least one other volatile compound produced besides CO_2. Consequently the method of immersing an isolated nerve directly into a solution to estimate CO_2 by measuring the rate of change of the hydrogen ion concentration might cause a very serious error, unles we can ascertain that not only the other gas has no influence upon H^+ produced by CO_2, but also that there are no other compounds diffusing in or out of the tissue in the solution which effects the reaction of the medium.

Digitized by Google

In view of this experience and considering the easy availability of standard indicator tubes now on the market for determination of the H^+ concentration, the author expected some one to report similar results, namely, that the increased metabolism accompanying stimulation cannot be detected by a direct indicator method. It is not surprising, therefore, to see an article (2) by A. R. Moore of Rutgers College in which such a negative result is reported; and to him all the credit for this rediscovery of the negative result obtained by very early workers should be given. It is, however, surprising indeed to see him dismiss this problem by saying that "*The nerve impulse does not depend upon processes leading to the production of carbon dioxide.*"

The present communication is, however, not to show whether or not an indicator method should be used for estimation of CO_2 in the nerve fiber, nor to consider all factors which are sufficient to explain why contradictory results were obtained with the indicator and our original CO_2 methods (3). It is, first, to prove that the nerve fiber gives off a basic substance simultaneously with an increased production of carbon dioxide and that this substance is most probably ammonia; and second, to consider the relationship between irritability and ammonia formation in the nerve under various conditions.

EXPERIMENTAL: *Part 1. Preliminary consideration.* If the sciatic nerve of a frog is placed in Ringer's solution, made slightly alkaline and colored with any weak acid indicator like phenolphthalein, sooner or later the fluid gives an acid reaction. If, therefore, we assume that the failure to detect the increase of CO_2 during the stimulation is due entirely to ammonia alone, we should expect that the maximum amount of ammonia production cannot be greater than that necessary to neutralize 8.7×10^{-7} grams of CO_2 for 10 mgm. of the nerve during 10 minutes of respiration using phenolphthalein as an indicator, since for the same units, a resting nerve gives off 5.5×10^{-7} grams and an activated nerve 14.2×10^{-7} grams CO_2 at ordinary room temperture, provided of course our previous estimation of CO_2 is correct.

Theoretically, 34 grams of NH_3 should neutralize 44 grams of CO_2, and 8.7×10^{-7} grams $\times \frac{34}{44} = 6.7 \times 10^{-7}$ grams of NH_3 should be required to completely neutralize just the amount of increased CO_2 produced during excitation. However, since NH_4OH is more highly ionized than H_2CO_3, the probability is that far less an amount of NH_3 than 6.7×10^{-7} grams will be able to maintain a definite level of H^+ concentration in presence of 8.7×10^{-7} grams of CO_2, which is the

Digitized by Google

amount increased in 10 minutes by 10 mgm. of the stimulated sciatic nerve of a frog, under ordinary conditions.

How far this hydrolysis of $(NH_4)_2CO_3$ will affect the turning point of the indicator will depend not only on the concentration of $(NH_4)_2CO_3$, and kind of indicators and the amount of free CO_2 present, but also upon the presence of other salts as well as on temperature. Since available data on these points are almost inapplicable to the condition under which we are working, the exact relationship between NH_3 and CO_2 in respect to maintenance of certain H^+ concentration in dilution approximately that of our problem is under separate investigation, the results of which will be published elsewhere in conjunction with Mr. L. S. Friedman.

The point of the foregoing consideration is, however, to show that the amount of NH_3 produced under the conditions stated might be far less than 6.7×10^{-7} grams and that any method for detection of the gas must necessarily be exceedingly delicate.

Part 2. Qualitative experiments. In the following experiments we shall see whether or not the living nerve gives off something else besides CO_2, and if so, we shall attempt to identify the nature of this compound. In these cases, we used more than 100 mgm. of fresh sciatic nerves of the frog, and let them respire more than 15 minutes.

a. Does the resting nerve give off something else than CO_2 when immersed in Ringer's solution?

Experiment 1. Tube i: 3 cc. Ringer + nerves; tube ii: 3 cc. Ringer.

When tubes i and ii are Nesslerized after a definite time of respiration, tube i will show a more intense yellowish color than ii. This suggests a possibility of NH_3 formation but not necessarily, since it is known that CO_2 and other compounds by virtues of forming precipitates or similar colors, might produce different colorations.

The following experiments may eliminate CO_2 factor.

Experiment 2. Tube i: 3 cc. Ringer + nerve; ii: 3 cc. Ringer.

At the end of respiration, 1 cc. of N/800 H_2SO_4 is added to each tube and placed in boiling water for 5 minutes. The obvious reason of this treatment is of course to boil off CO_2 without losing NH_3 if present.

Since under this condition tube i is still more intensely colored after Nesslerization than the control, it is certain that the living nerve when immersed in Ringer's solution, gives off something besides CO_2.

b. Is it ammonia? The experiments cited above do not rule out the possibility that the slight amount of neutral ammonium salt might have diffused out of the nerve or that some compound other than NH_3 might be diffused or produced from it.

Digitized by Google

1. If it is ammonia, it should be volatile; we ought to be able to absorb it out of the air by acid.

Experiment 3. Tube i: 1 cc. of N/800 H_2SO_4 + nerve (hanging without touching the solution); tube ii: 1 cc. of N/800 H_2SO_4.

At the end of respiration, the tubes are shaken without moistening the nerve and the tissue removed, and both of the tubes are immersed in boiling water for 6 minutes. To the bottom of cooled solutions, 1 cc. of Graves' reagent[2] is carefully introduced. Graves' reagent gives a white precipitate with ammonia, Tube i gives a faint white cloud at the junction of the 2 fluids. Tube ii gives no precipitate. The ring test thus performed shows conclusively that the compound is volatile and gives an insoluble complex salt with $NaHgCl_3$ in alkaline solution, exactly in the same manner as do NH_4 salts.

2. If this is ammonia, it should not only be volatile, but also should form a base. The base-forming property of this compound can easliy be demonstrated by the following experiments.

Experiment 4. Tube i: 1 cc. Indicator[3] + unstimulated nerve; ii: 1 cc. Indicator.

The tubes are corked and allowed to stand.

At the end of respiration these tubes are shaken and the tissue is removed. The tubes are then immersed open in boiling water for 6 minutes. If at the end of the boiling there is not a detectable difference in the color, then to each tube add alternately drop by drop of distilled H_2O kept in *ordinary glass* to each tube. In the course of adding this exceedingly weak alkaline solution, it will be noted that tube *i* which has had the nerve will decolorize first, then *ii*. Since this indicator will lose its pink color when H^+ concentration reaches pH = 5 to 6, it is evident that as less alkali needs to be added to tube i this compound given off by the nerve has a base forming property.

c. Does the activated nerve give off this compound more than the resting? In similar experiments as described above but by substituting for the control the tube containing a stimulated nerve, it can be demonstrated that the nerve when stimulated gives off more of this compound than the resting.

d. Is this an amine or ammonia? 1. The fact that this compound is volatile and forms a yellow complex salt with Nessler's reagent and a white precipitate with $NaHgCI_3$ (Graves' reagent), strongly suggests that it is NH_3 gas, but does not absolutely rule out the possibility of

[2] See page 525.

[3] See page 528.

Digitized by Google

its being one of the volatile amines. There are theoretically many ways by which we may be able to differentiate amines from NH_3, yet we have not succeeded in applying them satisfactorily to such a small concentration as that with which we are dealing. If, however, the quantitative data obtained by two methods based on entirely different chemical properties of the substance, agree within experimental errors, then the identity of this compound can easily be ascertained.

Thus by estimating basicity produced by this compound, we may calculate it on the basis of NH_3, and compare the results with those obtained by Nessler or Graves' methods using standard ammonium salt. If such results do not agree with each other, we may calculate the basicity on the basis of all volatile amines, and compare the results obtained from the other method using standard solution of various alkyl ammonium salts.

Considering the difficulty and large sources of error obtained by turbidity experiments with exceedingly minute amount of this substance, the results obtained by these two methods are satisfactorily concordant to show that it is ammonia. (See page 523.)

On the basis of this evidence, we shall from now on call this compound ammonia until we shall have evidence to the contrary.

Part 3. Quantitative methods.[4] The two well-known properties of NH_3, used in ordinary methods, can be applied for measurement of exceedingly minute quantities. With slight modification and a great deal of care, one can detect an amount of NH_3 gas as small as 0.000,000,1 gram either by means of converting it to a complex salt with Hg. (Nessler or Graves'), or by measuring the amount of base formed by the gas. Since, however, the presence of CO_2 will interfere with all the methods based on these properties (the basicity in the greatest degree), it is absolutely necessary to have a device to eliminate CO_2. It is equally essential to avoid a direct contact between the tissue and liquid in which NH_3 is to be estimated, on account of possible diffusion of neutral NH_4 salts or other nitrogenous extractives that would react with direct Nesslerization or Graves' reagents, and on account of the possible presence of buffers either diffused out of the tissue or present in the tissue itself that would mask the true basicity attributed to ammonia alone. The amount of NH_3 gas actually given off by the nerve can only be estimated by suspending the tissue over a very small amount of dilute acid, the concentration of which should be kept con-

[4] For checking up the method as well as certain experiments, the author is greatly indebted to Miss Olive Pearl Lee and Dr. H. Sugata.

Digitized by Google

stant not only for all the nerves and controls, but also for the standard in each set of experiments.

A. Ring test with Graves' reagent. A new precipitant for ammonia recommended by Graves[5] is just as sensitive as that of Nessler. When used as a ring test, moreover, we found that as small as 1×10^{-7} grams N in form of NH_3 can be easily detected. This formation of a ring depends, however, upon two factors, i.e., concentration of the acid and of NH_4. For example in N/400 H_2SO_4, 2.5×10^{-7} grams N per cc. is barely detectable, while in N/800 H_2SO_4, 1×10^{-7} grams N will form a white ring within 3 to 4 minutes.

By varying acidity, therefore, over which the nerve is to respire, and by determining how much NH_4 is necessary to form a ring in the acidity in which a positive ring was formed, and over which nerve was suspended, we can estimate how much NH_3 is produced from the given nerve during a given period of respiration.

There are two main difficulties with this method. First, it is exceedingly difficult to determine the absolute point at which ring formation occurs, the speed of which depends not only on the concentration of NH_3, acidity, but also temperature. Second, it involves maintenance of a series of standard acids whose blank ammonium contents must be kept constant. If this varies, there is no way of correcting an error without performing a series of elaborate quantitative estimations with the different ammonium standards in different concentrations of the acids. Although this latter difficulty can be eliminated to a great degree by making up for each experiment a series of standards of the ammonia in the same acidities which are used for absorption of NH_3 from the nerve, yet the amount of acidity reduced by the NH_3 has not been reckoned with the standard.

In addition to this, any quantitative method based on such a formation of a barely visible ring is impossible to make free from a personal factor. But for a quick qualitative-quantitative test to determine which gives more NH_3, this ring test with Graves' reagent is very convenient and reliable.

B. Nephelometer method. This is essentially Graves' method. Since, however, the amount of NH_3 is so small a minor modification is necessary. In this, the use of starch is omitted on account of the

[5] Graves' reagent is made up as follows: To 80 grams of NaCl, are added 130 cc. H_2O and 100 cc. of cold sat. $HgCl_2$ solution with shaking. When the salt is practically all dissolved, 70 cc. of sat. Li_2CO_3 are added slowly while shaking, so that no mercury oxide forms on the side of the flask.

Digitized by Google

fact that the precipitate is so little as to maintain almost permanent turbidity without the use of any protective colloid. In order to keep the turbidity at the possible maximum, the dilution with NH_3-free H_2O is omitted. Ordinarily, for each cubic centimeter of N/800 H_2SO_4 over which the nerve is suspended, 10 cc. of Graves' reagent are added and the resulting turbidity is matched against the standard which contains 10 cc. of the reagent and 1 cc. of the standard $(NH_4)_2SO_4$ made up in N/800 H_2SO_4.

Although this method should be theoretically far superior to that of the ring test, the accurate matching of this exceedingly small turbidity is exceedingly difficult with the best nephelometer on the market even to the best and most experienced person. Any effort to increase the amount of turbidity by using a larger number of the isolated nerves is apt to introduce very serious physiological errors.

In addition to these, the method is so tedious on account of necessity of having perfectly clean and dry tubes ready, that it is impossible to run the quantitative estimations more than two dozen a day without having two or three dozens of nephelometer tubes that are uniform in all regards. Expense has precluded obtaining any such number. Unless for the purpose of identifying NH_3 by checking the result with the titration method, we have discarded the use of this method for a routine determination of NH_3.

C. Titration method: 1. Principle. By far the most satisfactory method, we found, is a titration method. The method itself has no claim of originality. The nerve is suspended over a definite quantity of an acid which contains a proper indicator. At the end of the respiration, CO_2 is driven off by immersing in boiling water 6 minutes, and the remaining acidity is titrated drop by drop with an alkali.

In spite of simplicity of the principle, however, there are many necessary details and precautions, without which the method is not dependable.

2. Apparatus. No special apparatus is necessary except that all, unless otherwise stated, should be made of Pyrex glass. Respiration and titration are performed in ordinary 6 inch Pyrex test tubes. One of the greatest sources of error and difficulty will be with these test tubes. For a successful experiment, it is convenient to have over 100 of the Pyrex test tubes and have them ready according to the following procedures:

These tubes are boiled in about 10 per cent H_2SO_4 for one hour and washed thoroughly with ordinary distilled water several times. Finally

Digitized by Google

these are again rinsed at least twice in ordinary distilled water, filling the tube to the top in each rinsing. These are then boiled in ordinary distilled water for one hour twice, and finally boiled in NH_3-silicate free[6] H_2O for one hour.

After being heated in a hot oven for 2 hours, the tubes are directly transferred in a con. H_2SO_4 desiccator. The tubes thus prepared should be tested for their cleanness in the following way.

When a solution of phenolphthalein made alkaline so that it is just perceptibly pink is added to the tube, it should not be decolorized in the cold. The faint pink color of methylene blue-methyl red (see p. 528) should stay permanent in the test tube without heating. When 1 cc. of the same indicator-acid solution is heated in this tube for 6 minutes in boiling water, the pink color should not be intensified. On the other hand, if this color changes to deep yellow the tube should be discarded.

All the tubes which stood a successful test were rinsed with redistilled H_2O and boiled again in redistilled H_2O once, and finally boiled in water free from ammonia and silicate for one hour. After being heated in a hot oven for 2 hours, they are directly transferred in a con. H_2SO_4 desiccator, ready for the experiment.

The tubes once used successfully for ordinary respiratory experiment should be subjected to similar cleaning as described in the last paragraph, i.e., they need not be boiled again in the strong acid.

In spite of all these precautions, one often finds a tube which is contaminated with alkali or acid. In the course of the experiment, one may also desire to add an excess of alkali or acid for various reasons to a tube. Such tubes should be set aside, separate from the regular tubes, and be subjected to more careful washings than the tubes which are used for routine quantitative experiments.

3. Solutions: a. Water free from NH_3 and silicate. Ordinary method of preparation of NH_3-free-H_2O is employed in preparing this water, care being taken, however, to use Pyrex glass in every part of the distillation apparatus where water or its vapor comes in contact with it. Thus it is recommended to use Pyrex distilling flask, a condenser whose inside tube is made of Pyrex, and receptor and adapter, all made of the same material. This NH_3-free-H_2O freed as much as possible from silicate is kept in a Pyrex bottle or flask tightly stoppered with paraffined cork.

[6] See last paragraph, this page.

Digitized by Google

b. Indicators: 1. Methylene blue solution, 0.5 gram of methylene blue, special,[7] is dissolved in 200 cc. NH_3-silicate-free H_2O. A 0.025 per cent solution is made from this stock solution by diluting 10 cc. to 100 cc. with NH_3-free H_2O.

2. Methyl red. Methyl red, special, recrystallized from alcohol, is saturated in 50 per cent of redistilled alcohol at room temperature.

3. To make methylene blue-methyl red (MB-MR) indicator. For 1 cc. of 0.025 per cent of methylene blue, add 10 cc. of methyl red solution.

4. Ordinary alcoholic solution of phenolphthalein.

c. Standard solutions: 1. N/20 H_2SO_4 solution, made up from a standard acid by diluting with NH_3-free H_2O, is kept in several small bottles well protected from the air.

2. Standard alkaline solution. The error of the experiment will depend a great deal upon the concentration of the standard alkali solution. If it is too low, the end point will not be sharp. If it is too high, the NH_3 can not be detected. Any alkaline solution which contains available alkalinity of N/10,000 or about, is satisfactory, that is when 0.1 cc. N/20 H_2SO_4 + 1.2 cc. MR-MB is made up by this solution to 100 cc., the pink color should disappear when immersed in boiling water for 6 minutes. If such alkali solution is completely ionized, H^+ should correspond to between pH 8–9, just the turning point of phenolphthalein, and of course way up on the alkali side of MB-MR. When, however, such a solution contains a trace of silicates, even if it is decidedly acid to phenolphthalein, the solution will often be too strongly alkaline for our purpose. Therefore as long as we are not sure of absence of silicate in the water, the ideal method of preparation of this solution on the basis of free H^+ concentration alone as determined by indicators will not be safe unless it is checked by a titration in a manner described below.

The method by which one can prepare the satisfactory concentration of alkaline water is as follows. Several liters of NH_3-silicate-free H_2O is poured into a large Pyrex flask and a few drops of phenolphthalein solution added. After addition of each drop of N/20 NaOH, the

[7] Presence of proper amount of methylene blue is very important for detecting the end point. Since we all know that no two brands of methylene blue on the market are the same, we have specified the amount on the basis of methylene blue, special, prepared by Coleman and Bell, Norwood, Ohio. If one prefers to use other brands, it will be necessary for him to determine proper concentration of the indicator to use.

Digitized by Google

bottle is tightly stoppered with paraffined cork and shaken until a faint but distinct pink color persists. Then enough N/20 H_2SO_4 is added to barely decolorize the pink color. The flask is filled with a Pyrex syphon; the inlet of air is protected with 15 per cent NaOH and concentrated H_2SO_4. The tip of the syphon is also provided with cork to which a Pyrex test tube can be inserted to protect against diffusion of the gases through the tip.

This solution should be tested out for its proper alkalinity in the following way.

To a Pyrex flask marked at 100 cc., 0.1 cc. of N/20 H_2SO_4 and 1.2 cc. of MB-MR are added using Pyrex pipettes. The volume is made up to 100 cc. mark with the alkaline solution just described. If the resulting solution is greenish yellow, it is apt to be too strongly alkaline. It should be colored faintly pink in cold, but should become a pale greenish yellow when a few cubic centimeter of this are placed in the Pyrex test tubes, previously tested, and immersed in boiling water for 6 minutes.

When 0.2 cc. of N/20 H_2SO_4 is taken and tested in the same way, the color should be faintly pink after immersion in the water for the same period of time. In other words the concentration of the alkali should be somewhere around N/10,000.

3. Standardization of the standard alkali. This approximately right alkali solution is standardized as follows.

To each of four Pyrex flasks marked at 100 cc. various fractions of cubic centimeter from 0.5–0.2 cc. of N/20 H_2SO_4 accurately measured with Pyrex pipette and 1.2 cc. of MB-MR, measured also with Pyrex pipette, are added, and made up to the volume with the alkali solution. One cubic centimeter of each is measured off by Pyrex pipette from each flask into a clean Pyrex test tube, previously tested, and immersed in boiling H_2O. At the end of 6 minutes, each tube is titrated drop by drop with the same alkaline solution. The typical result of titration of a particular standard solution we made up as shown in table 1.

The average of these determinations, then, shows that 7.2 drops of alkali solution were necessary to neutralize 1 cc. of N/20,000 H_2SO_4, since each flask contains increment increase of 0.1 cc. of N/20 per 100 cc. and since we took for titration 1 cc. only. On the basis of this titration, each drop of the alkali is equivalent to 1/7.2 cc. of N/20,000 alkalinity, which in terms of NH_3 corresponds to 0.000,000,12 gram.

It should be noted that in the above calculation, we have not ignored the effect of the indicator itself, but have eliminated it on account of the

Digitized by Google

fact that each cubic centimeter of the acid we took contained exactly the same amount of indicator, and by subtracting number of drops from the one above, the acidity due to indicator itself is canceled.

TABLE 1

TUBE	CUBIC CENTIMETERS OF N/20 N_2SO_4	CUBIC CENTIMETERS OF MB-MR	MADE UP WITH THE ALKALINE SOLUTION	NUMBER OF DROPS REQUIRED TO NEUTRALIZE 1 CC. OF THE SOLUTION	DIFFERENCE FOR EACH TUBE
					drops
A	0.5	1.2	100	22	7.0
B	0.4	1.2	100	15	7.5
C	0.3	1.2	100	7	7.0
D	0.2	1.2	100	0	
Average drops					7.2

From the same data, however, we may calculate the acidity contributed by the indicator, as shown in the following table.

TABLE 2

TUBE	CUBIC CENTIMETERS OF N/20 H_2SO_4	CUBIC CENTIMETERS OF MB-MR USED	MADE UP WITH THE ALKALINE SOLUTION	NUMBER OF DROPS OF THE ALKALINE SOLUTION NECESSARY TO NEUTRALIZE 1 CC. OF THE SOLUTION	NUMBER OF DROPS CONTAINED IN 1 CC. PIPETTE USED TO MEASURE THE ACID-MB-MR	SUM OF DROPS	NUMBER OF DROPS REQUIRED TO NEUTRALIZE 1 CC. N/20000
A	0.5	1.2	100	22	18	40	40/5 = 8.0
B	0.4	1.2	100	15	18	32	32/4 = 8.0
C	0.3	1.2	100	7	18	35	25/3 = 8.3
D	0.2	1.2	100	0	18	18	18/2 = 9.0

Average for first three = 8.1 drop. Average for all, including the last which was already decolorized before titration = 8.3. Average of these two = 8.2; 8.2 − 7.2 = 1 drop = the amount of alkali required to neutralize 1.2/100 cc. of MB-MR indicator, the amount of indicator contained in 1 cc. of MB-MR-acid.

If we can ignore the acidity contributed by the indicator, the standardization of the alkali solution can be done by a simple method. For instance, take 0.5 cc. of N/20 H_2SO_4 and 1.2 cc. of MB-MR, and dilute it to 100 cc. with solution to be standardized. Titrate 1 cc. of this solution according to the method described above. Add the number of drops contained in 1 cc. of the pipette used for titration, to the

Digitized by Google

number of drops of the alkali required to neutralize the acid-MB-MR. The sum of the drops divided by 5 will be equivalent to 1 cc. of N/20,000. The general formula for this calculation is as follows:

$$\text{One drop} = \frac{1}{\frac{(a+b)}{c}} \times 0.000{,}000{,}85 \text{ gram } NH_3$$

where a = number of drops required to neutralize 1 cc. of the solution.

b = number of drops contained in each cubic centimeter of the pipette used for titration.

c = number of cubic centimeters of 1/200 cc. H_2SO_4 (number of tenths of cubic centimeter of N/20 H_2SO_4) used to make the solution and 0.000,000,85 gram is amount of NH_3 contained in 1 cc. of N/20,000 NH_4OH solution.

If one uses always the same fresh indicator, and is sure of the amount of acid it contributes, then the following formula can be used, since 1.2/100 cc. of MB-MR does neutralize 0.000,000,02 gram of NH_3.

$$\text{One drop} = \frac{1}{\frac{(a+b)}{c}} \times 0.000{,}000{,}85 \text{ gram } NH_3 + 0.000{,}000{,}02 \text{ gram.}$$

Under strictly ideal experimental conditions, there are 3 factors which will determine the error of determination: a, correct standardization of the alkali; b, any factors which influence size of the drop, and c, end point. The first two factors concern the accurate estimation of the amount of alkali taken. If one wishes, therefore, to be exceedingly accurate, instead of measuring it by drops, use of a very small Pyrex pipette, accurately calibrated to hundredths of a cubic centimeter will narrow the limit of error. The effect of temperature on the number of the drop should, of course, be always remembered.

Although the end point is sharp within ½ drop of the alkali we ordinarily use, the error will be enormously great if the number of total drops of the alkali used is very small. It is highly desirable for one to decide what point is the end and use that criterion for all the experiments, both standardizations of the alkali, and titration of the remaining acid after the respiration. The point of change from pink to faint greenish yellow is the most sharp. Many unnecessary errors will be eliminated if one uses a control tube in which the same amount of the indicator is placed and which has the greenish yellow color to be compared.

During titration, care should be taken not to let the alkali drop touch the side of the tube, but to let it drop directly into the solution.

Digitized by Google

4. Preparation of MB-MR-acid solution. If we know the exact concentration of the alkali, theoretically any strength of the acid should be satisfactory, provided we run a control tube containing exactly the same amount of the acid. Like the Kjeldahl titration, however, it is best to have such an acidity that each cubic centimeter of the control should not require more than 1 cc. of the standard alkali solution. That is, if one used the alkali solution to dilute the N/20 H_2SO_4 to 100 cc. with MR-MB, the original acidity should be slightly more than twice as strong as the standard alkali. If too strong acid is used, the indicator is so diluted during titration, that the end point may be a little obscure. If too weak acid is used, the acid may be already neutralized by the ammonia before titration. Thus according to the type of the tissue, length of the respiratory period and weight of the nerve, the acidity may have to be altered after a preliminary experiment. For ordinary 15 minutes' respiration with 2 sciatic nerves, a MB-MR-acid solution prepared in the following ratio is found to be satisfactory: 0.4 cc. N/20 H_2SO_4 + 1.2 cc. MB-MR are made up to 100 cc. with the standardized alkali.

D. Detailed method of estimation of the NH_3 given off by the nerve. A typical experiment to estimate simultaneously the amount of NH_3 given off by resting and stimulated nerve is as follows: Four clean test tubes are placed in the rack, each tube being provided with a paraffined cork, having 2 electrode attachments. Prepare the indicator-acid solution, by taking 0.4 cc. N/20 H_2SO_4 + 1.2 cc. MR-MB and making it up to 100 cc. with the standard alkali solution, using a flask and both pipettes made of Pyrex. By means of another Pyrex pipette, 1 cc. of each of the indicator-acid solution is placed in the above tubes, and tightly stoppered with the cork. Two pairs of the sciatic nerves are quickly isolated from two frogs, and immersed in each of two dishes containing Ringer's solution. One out of each pair is taken together, blotted and weighed. The remaining two are also likewise weighed. After the adhering liquid is carefully removed by means of filter paper, each set of nerves is placed in tubes S and R, respectively, the corks are replaced and stimulation by a weak induction shock is applied to the nerves in tube S, recording the time at which the respiration begins. The stimulus should be so weak as to be barely perceptible to the tongue. Tubes c_1 and c_2 are controls. All the tubes are shaken gently once or twice during experiment, care being taken not to let the liquid touch the nerves. The best way is to shake the rack gently without touching each tube with finger, in order to avoid temporary rise of temperature of the tube.

Digitized by Google

At the end of 15 minutes' respiration, each tube is shaken before the tissue is removed. After the stopper is removed, the upper third of the tube is wiped with filter paper so as to remove any liquid of the tissue which might have been left on the side of the tube.

The four tubes are placed in a basket which is hung in boiling H_2O, and which is placed at least one inch above the bottom of the beaker in which the water is boiling. At the end of 6 minutes, the tubes are removed and titrated while still hot with the standard alkali. The standard alkali can be introduced either from Pyrex buret, syphon or pipette, provided we have standardized value of each drop of our alkali with the same apparatus.

The different amounts of drops of alkali required to neutralize control tubes and tubes containing the nerves when multiplied by number of grams of NH_3 equivalent to one drop of the alkali will indicate the amount of NH_3 given off by the nerves during that period of respiration.

The record given in table 3 will give an idea of the details of the method used in above experiments.[8]

TABLE 3

Exper. 8. 12/24/1920. Two frogs, Rana pipiens (♂, 22 cm.; ♂, 20 cm.) of lot VI (arrived at the laboratory on 11/12/1920) decerebrated simultaneously at 10:57 a.m. Respiration at 24°C.

Tissue			Number of tubes*	Stimulation	Respiration (15 minutes)		Drops of alkali necessary to neutralize the remaining acid	Amount of acid neutralized by NH_3	Total NH_3 given off	NH_3 given off by 10 mgm. of the tissue during 10 minutes
Kind	Isolated at	Weight of			From	To				
	a. m.	*mgm.*			*a. m.*	*a. m.*		*drops*†	*gram*	*gram*
2 sciatics	10:58	80	S	Yes	11:09	11:24	7	7	0.98×10^{-6}	0.81×10^{-7}
2 sciatics	10:59	80	R	No	11:09	11:24	11	3	0.42×10^{-6}	0.35×10^{-7}
		0	C_1		11:10	11:25	14			
		0	C_2		11:10	11:25	14			

* Each tube contains 1 cc. of MB-MR-acid solution.

† Each drop of the alkali is equivalent to 0.14×10^{-6} gram NH_3.

[8] Immediately after smoking, the breath contains appreciable amounts of base-forming substances. It is always safer, therefore, for a smoker to wash his mouth thoroughly before he uses pipettes.

Digitized by Google

RESULTS: *1. Resting nerve.* The results obtained by the last method show that the resting nerve gives off exceedingly small, but quite definite amounts of ammonia. The results given in table 4 were taken from experiments conducted with two bundles of sciatic nerves (70–100 mgm.) taken from frogs ranging in size of 19 to 24 cm. in length, under ordinary range of temperature variation (20–24°C.). The gas was collected exactly during 15 minutes of respiration starting it at about 10 minutes after the animals were decerebrated. The other nerves of the same frogs were stimulated and the NH_3 collected simultaneously with that of resting nerves.

In the following table, NH_3 production from stimulated and non-stimulated nerves are given to show the range of variation. If one compares two nerves of the same animal, we have no difficulty in showing increased NH_3 production during stimulation, but the actual amount of the gas given off by nerves of different animals varies considerably. Although these variations might be due to an error of the method which is necessarily great, yet we have evidence to show that there are other experimental and physiological conditions which have a great influence upon NH_3 production. An investigation is now under way in which these influences are more carefully studied. Until we shall know more about these factors, it is of prime importance to determine the amount of NH_3 given off by the unstimulated nerves of the same frog and compare it with the NH_3 produced by the other half under various conditions whose influence one wishes to investigate.

On the basis of 10 mgm. of the nerve for 10 minutes of respiration the unstimulated sciatic nerves of frogs (*Rana pipiens*) give 0.32×10^{-7} grams NH_3 on the average.

It is interesting to note that this amount of NH_3 corresponds to approximately $\frac{1}{17}$ of the weight of CO_2 given off under approximately the same conditions, and therefore for each mol. of CO_2, 1/6.5 of a mol. of NH_3 is given off, i.e., $\frac{1}{13}$ of equivalent weight of CO_2.

2. Stimulated nerve. In these experiments we used the ordinary method of electrical stimulation by weak induced current only, similar care being taken as described in our earlier work on CO_2 production. The remaining 2 of the nerves of the frogs, used in the experiment with the resting nerve were taken and stimulated side by side with unstimulated nerve, thus maintaining physiological and other experimental conditions as constant as possible.

The average amount of NH_3 by stimulated nerves is 0.68×10^{-7} grams as expressed on the basis of 10 mgm. tissue and 10 minutes'

Digitized by Google

respiration. Thus average amount of NH_3 given off during stimulation is approximately twice that of the resting nerve. This corresponds closely to the average increase of CO_2 in similar nerves during stimulation which was found to be 2.4 times that of the resting nerve.

TABLE 4

NUMBER OF EXPERIMENT	TEMPERATURE	WEIGHT OF NERVE	TIME ELAPSED FROM DECEREBRATION OF FROGS TO BEGINNING OF RESPIRATION	DURATION OF RESPIRATION	STIMULATION	TOTAL NH_3 GIVEN OFF	AMOUNT OF NH_3 GIVEN OFF, CALCULATED ON BASIS OF 10 MGM. OF NERVE AND 10 MINUTES OF RESPIRATION
	degrees C.	*mgm.*	*minutes*	*minutes*		*grams*	*grams*
2	23.0	105	9	15	−	4.0×10^{-7}	0.254×10^{-7}
		102	9	15	+	7.0×10^{-7}	0.457×10^{-7}
3	23.5	80	9	15	−	3.6×10^{-7}	0.30×10^{-7}
		80	8	15	+	10.8×10^{-7}	0.90×10^{-7}
4	20.0	90	11	15	−	4.8×10^{-7}	0.355×10^{-7}
		85	11	15	+	9.0×10^{-7}	0.705×10^{-7}
5	20.2	68	13	15	−	2.4×10^{-7}	0.235×10^{-7}
5a	20.0	95	13	19	−	5.6×10^{-7}	0.31×10^{-7}
		88	13	19	+	14.0×10^{-7}	0.83×10^{-7}
6	20.2	78	11	15	−	4.9×10^{-7}	0.419×10^{-7}
		72	11	15	+	7.0×10^{-7}	0.642×10^{-7}
8	24.0	80	11	15	−	4.2×10^{-7}	0.35×10^{-7}
		80	11	15	+	9.8×10^{-7}	0.81×10^{-7}
9b	23.0	80	13	15	+	4.8×10^{-7}	0.4×10^{-7}
Average for non-stimulated nerve							0.32×10^{-7}
Average for stimulated nerve							$0\ 68 \times 10^{-7}$

3. NH_3 production following stimulation of the nerve. In these experiments, a series of test tubes containing exactly the same amount of MB-MR-acid solution was prepared in a group of 4. Two nerves were placed in the first tube and the other two in the next, the remaining two of the group acting as controls. The nerves in the first tube were stimulated for 15 minutes. At the end of 15 minutes, after usual procedure, both stimulated and unstimulated nerves were transferred into

Digitized by Google

corresponding tubes of the second group, no more stimulation being applied to the stimulated nerve during subsequent respiration.

The results of titration of each of these later tubes show that during the first 15 minutes following stimulation, the nerve gives off always more than the unstimulated nerve. Under ordinary condition, the amount of NH_3 produced by the unstimulated and the stimulated nerve during post-stimulation becomes equal at the end of 45 to 60 minutes. We have not yet succeeded in establishing the exact condition under which the two nerves give exactly the same amount of NH_3 after a definite time. One thing is certain, however, that an increase in NH_3 production due to stimulation keeps on for some time after the external stimulation is stopped.

Whether this increased NH_3 production during post-stimulation is due to an increase of NH_3 formation due to hyper-irritable condition of the nerve during post-stimulation, diffusion of preformed NH_3 produced during previous stimulation, or due to release of NH_3 by virtue of gradual oxidation of lactic acid, which if formed should tend to hold a part of NH_3 formed during stimulation, can not be decided without further experiments.

4. Effect of injury. We have shown before that the nerve, when mechanically injured, gives off more CO_2 than the uninjured, and attempted to explain the fact by considering the traumatic injury to be analogous to an extreme form of stimulation. Since a stimulated nerve gives off more NH_3 in a similar manner to CO_2, we expected to produce more NH_3 in the nerve. The results were, however, diametrically opposite. The crushed nerve not only does not give off more NH_3 than the resting, but gives far less, the amount varying from $\frac{1}{2}$ of that of the resting to none at all.

The mere fact that the nerve gives off less NH_3 under these conditions than uninjured and unstimulated nerve, does not necessarily, of course, rule out the possibility of an increase NH_3 production during trauma. On the contrary, in spite of these facts, there are reasons for believing that an actual formation of NH_3 is increased under all forms of stimulation.

Whether or not the nerves produce lactic acid under certain conditions has not been experimentally settled. If the nerve behaves similarly to muscle, then during trauma even if NH_3 might have been formed, we shall not be able to detect it by our present method unless such acid is removed by either being converted to a neutral substance or oxidized away. In case of muscle, however, Fletcher and Hopkins

Digitized by Google

(5) have shown definitely that the lactic acid formed during trauma can not be removed in the manner that happens to lactic acid produced during functional activity.

In the case of ammonia formation in muscle we can demonstrate these two different modes of behavior of lactic acid. As will be shown in another article, both injured muscle and muscle fatigued by successive stimulation give off practically no NH_3, but during recovery process, fatigued muscle will gradually give up NH_3, but not the injured muscle even if we let them "respire" more than 2 hours.

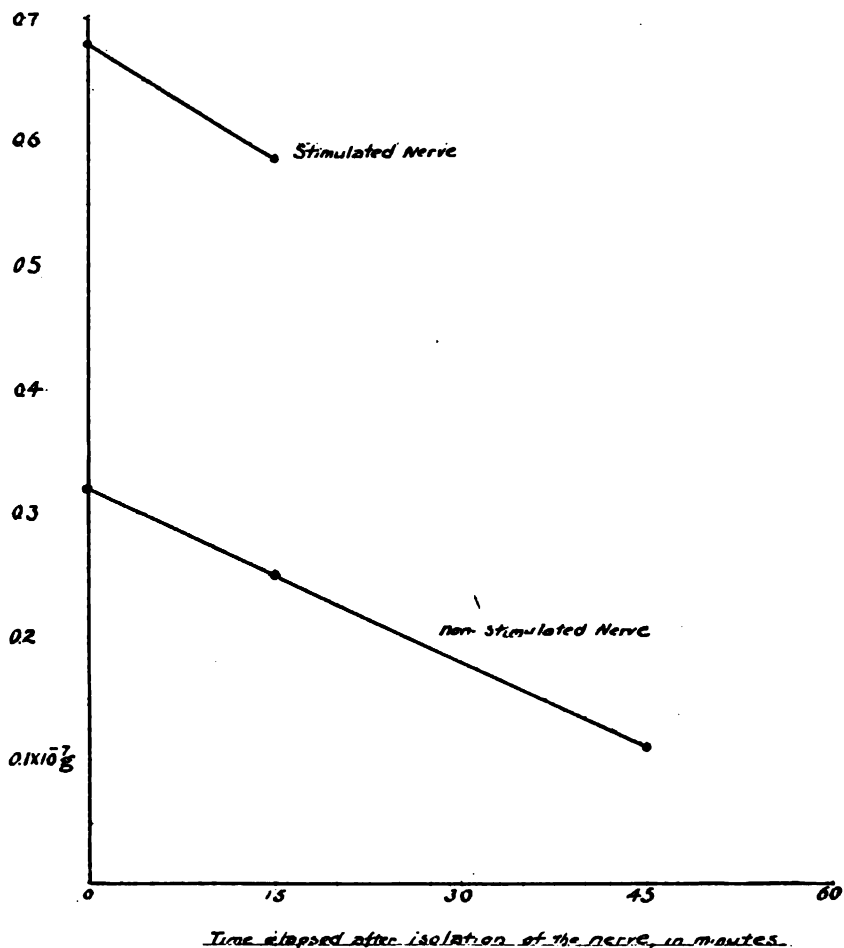
In the nerve, during a long period of respiration, the injured nerves seem to give up a part of NH_3. Whether this indicates formation of lactic acid in injured nerve, which holds NH_3, but which gives off a part of it later by partial oxidation of the acid unlike that of injured muscle or it means that traumatic injury was not complete and does not completely eliminate formation of NH_3 in the nerve, or that a certain amount of NH_3 keeps on coming independently of physiological process is a question to be determined more in detail.

5. Effect of standing on nerve NH_3. A knowledge of NH_3 production in the nerve during successive intervals after isolation from the animal is necessary in order to know the source of the gas. Gad-Anderson (6) has shown that muscle urea is spontaneously decomposed into NH_3 during post-mortem change, and certain bacterial or other decomposition is known to set in at a surprisingly early state of post-mortem period, such as histamine formation. Either one of these processes might give rise to NH_3 gas formation in a comparatively fresh tissue.

In general, isolation of a tissue from a body produces two opposing phenomena. From this point on, the survival physiological process gradually descends to zero, but post-mortem change gradually ascends to a certain climax. Whether the NH_3 we measured here is formed by survival physiological process or due to post-mortem phenomena unrelated to process in normal body, will be decided by the nature of the curve of NH_3 production during successive periods after the nerve is isolated from the body.

The gradual decrease of NH_3 production shown in the following chart, shows that NH_3 production in the nerve must be due to physiological process. We have not yet extended our observation on a large interval to see just when the physiological process reaches a minimum and to compare this point to the death point of the nerve determined by irritability and CO_2 method.

Digitized by Google



Curve 1

Summary of quantitative results. The summary of quantitative estimation of NH_3 produced by the nerve under various conditions is given in table 5.

6. *Quantitative results with nephelometer.* In order to check the results obtained by the method based on base-forming property of gas, a nephelometric determination was made. N/800 H_2SO_4 is used to

Digitized by Google

TABLE 5

Summary of NH_3 production from the sciatic nerves of frog, Rana pipiens, under different conditions

CONDITIONS	RESPIRATION PERIOD	AVERAGE AMOUNT OF NH_3 GIVEN OFF, CALCULATED ON BASIS OF 10 MGM. OF THE NERVE AND 10 MINUTES RESPIRATION	TEMPERATURE
		grams	*degrees C.*
Resting nerve......	15 minutes immediately after*	0.32×10^{-7}	20-24
	Next 15 minutes	0.25×10^{-7}	20-24
Stimulated nerve ..	15 minutes immediately after	0.68×10^{-7}	20-24
	Next 15 minutes	0.59×10^{-7}	20-24
Crushed nerve......	15 minutes immediately after	0 to 0.15×10^{-7}	20-24

* "immediately" is used for those cases when the respiration was started within 7 to 10 minutes after decerebration of the animal.

TABLE 6

NUMBER OF EXPERIMENT	TEMPERATURE	WEIGHT OF NERVE	TIME ELAPSED FROM DECEREBRATION OF FROGS TO BEGINNING OF RESPIRATION	DURATION OF RESPIRATION	STIMULATION	TOTAL NH_3 GIVEN OFF	AMOUNT OF NH_3 GIVEN OFF CALCULATED ON BASIS OF 10 MGM. OF NERVE AND 10 MINUTES OF RESPIRATION
	degrees C.	*mgm.*	*minutes*	*minutes*		*grams*	*grams*
AN 1	25.0	101	11	20	+	5.3×10^{-7}	0.26×10^{-7}
		103	11	20	−	3.8×10^{-7}	0.18×10^{-7}
AN 2	23.0	123	11	15	+	4.6×10^{-7}	0.25×10^{-7}
		141	11	15	−	3.8×10^{-7}	0.18×10^{-7}
AN 3	19.0	86	9	15	+	4.6×10^{-7}	0.35×10^{-7}
		87	9	15	−	2.8×10^{-7}	0.21×10^{-7}
AN 11	23.0	84	16	20	+	3.3×10^{-7}	0.20×10^{-7}
		87	16	20	−	2.6×10^{-7}	0.14×10^{-7}
AN 12	22.5	101	12	20	+	4.4×10^{-7}	0.22×10^{-7}
		90	12	20	−	2.6×10^{-7}	0.14×10^{-7}
AN 14*	24.0	103	12	20	+	6.4×10^{-7}	0.31×10^{-7}
		103	12	20	−	4.4×10^{-7}	0.21×10^{-7}
Average for non-stimulated nerve							0.17×10^{-7}
Average for stimulated nerve							0.27×10^{-7}

* Determined by Nessler.

Digitized by Google

absorb the gas, and treated with Graves' reagents as described on page 525. Both the Kober and our own modification of Dubosque colorimeter were used to determine resulting turbidity. On account of difficulty of the method, large numbers of readings were necessary for each experiment and our data are not extensive. The results obtained by this method are given in table 6.

In the sense of ordinary quantitative analysis, these two results shown in tables 4 and 6 cannot be said to be in a close agreement. Unfortunately like most so-called super-michrochemical methods, our method is subject to a much larger per cent of error than that which ordinary quantitative accuracy permits. It is highly probable that in our method there may be more than one error which is common to all our determinations. The absolute amount of NH_3 recorded here will no doubt be revised by some who will devise a more accurate method. In spite of this, however, we are quite certain that the relative amount of NH_3 gas produced by the nerve under various conditions will stand regardless of any method, within physiological variation.

Considering, therefore, the amount of the gas we are dealing with, these data obtained by methods based on entirely different chemical properties are close enough to show that the gas is ammonia.

CONCLUSION

However curious it may seem, the facts are that the nerve undergoes chemical reactions in which both acid-forming and base-forming substances are produced, and that the increase of CO_2 production during stimulation is accompanied with an increase of NH_3 production. Although the physiological and biochemical significance of these facts has not yet been investigated, they raise many interesting questions.

Where does NH_3 come from? We have endeavored to show that it is neither produced by bacterial decomposition nor from urea. Considering the minuteness of its amount, one might naturally suppose that inasmuch as the blood always contains a little NH_3, the nerve might receive it from the blood and retain it in an amount which will be in direct equilibrium with the blood, or lymph, and that this NH_3 will gradually diffuse out to a medium from which it is constantly removed. That this plausible explanation will not hold will be shown in later papers where we shall present evidence to show that NH_3 production from the different tissues is not the same, but varies within a large range, and that these variations are not proportional to anatomical variation, but due to some other physiological and biochemical factor.

Digitized by Google

Thus the process of elimination leads us to the speculation that this NH_3 must come from protein directly.

What becomes of it? According to the estimate made by Professor Donaldson,[9] an average adult human being weighing 150 pounds has 1,620 grams of total nervous tissues. If these tissues give off NH_3 in the same ratio as that of the sciatic nerve of the frog, we see that the daily output of NH_3 will amount to approximately 0.7 gram with corresponding variation during stimulation. Since the daily output of a normal man usually does not go beyond 1 gram of NH_3 and some other tissues also give off the gas in different degrees, and since by external factor alone one can almost completely abolish NH_3 output, even this entirely unqualified calculation tells us that the nerve NH_3 must be converted either entirely or partly into something else. It is not extraordinary speculation to consider that it is urea into which this ammonia is converted. Since no constituent of the urine is more variable than the urea, if NH_3 does go into urea, a variation of NH_3 production due to the changes in the nervous activity might easily be lost sight of.

How far this NH_3 influences acid-base balance in the body, and at what point it is converted to urea or something else can not even be speculated upon without further experimentation such as determination of the NH_3 production from the other tissues as well as an accurate determination of the NH_3 in the blood—a procedure which is one of the most difficult and unreliable of biochemical analyses.

What relationship has NH_3 with the irritability of the nerve? Does the fact that stimulation increases NH_3 production, suggest a decrease of NH_3 production during anesthesia? Muscle gives off far less am-

[9] Personal communication. For a man weighing 150 pounds, and who is 68 inches tall, his estimate of the total nervous system is as follows.

	grams
Brain	1400
Spinal cord	27
Sympathetic	30
Cranial nerves	12
Spinal nerves	151
Total weight	1620

Except weight of sympathetic which he considers more or less as a guess, these calculations are based on accurate anatomical data (8), (9), (10). For this information and many other suggestions, the author is greatly indebted to Prof. H. H. Donaldson.

Digitized by Google

monia than the nerve. Doctor Mathews suggested that this fact might be responsible for the extreme sensibility of muscle and relative immunity of the nerves to ammonia. Is there any relationship between NH_3 production and fatigue in view of the fact that the ratio between NH_3/CO_2 in activated nerve is far greater than that of the contracting muscle? What will be the NH_3 production from the nerve during prolonged refractory periods which are supposed to be more susceptible to a continued activity?

The accumulation of insoluble calcium salts at one point must be intimately concerned with a metabolism which forms bases. Drew (7) has shown that denitrifying bacteria can precipitate out $CaCO_3$ from sea water. In the physiological process of calcification and the pathological cases of softening of bone as well as pathological process of calcification, ammonia production in the tissues must be a dominant factor. This supports the idea that conditions like osteomalacia may be intimately related to disturbances in protein metabolism. Experiments to determine NH_3 production in the bone under different conditions will test these hypotheses.

In a series of papers on studies of alkaligenesis in the tissue, we hope to be able to answer some of these questions. Meanwhile we should emphasize the fact, as shown elsewhere (3), that any method which attempts to measure the small increase of CO_2 in the activated nerve should not ignore this exceedingly small but definite amount of NH_3 which is simultaneously formed in the nerve fiber.

SUMMARY

1. Evidence is given to show that resting nerves give off exceedingly minute quantities of a volatile base-forming substance which during stimulation is greatly increased, and that this substance is probably ammonia.

2. Methods are described by which we can measure NH_3 as small as 0.000,000,1 gram.

3. Quantitative data are given to show NH_3 production by the nerve under various conditions.

4. Various questions were raised concerning the rôle of NH_3 in the general physiological problem.

Digitized by Google

BIBLIOGRAPHY

(1) Tashiro: This Journal, 1913, xxxii, 107.
(2) Moore: Journ. Gen. Physiol., 1919, i, 613.
(3) Tashiro and Hendricks: Proc. Amer. Soc. Biochem., 1921, v, 15.
(4) Graves: Journ. Amer. Chem. Soc., 1915, xxxvii, 1171.
(5) Fletcher and Hopkins: Journ. Physiol., 1906–7, xxxv, 261, 288.
(6) Gad-Anderson: Journ. Biol. Chem., 1919, xxxix, 267.
(7) Drew: Carnegie Inst. Washington Publ. no. 182, 1914, 21.
(8) Donaldson and Bolton: Amer. Journ. Psychol., 1891, iv, 224.
(9) Donaldson and Davis: Journ. Comp. Neurol. and Psychol., 1903, xiii, 19.
(10) Ingbert: Journ. Comp. Neurol. and Psychol., 1904, xiv, 209.

Digitized by Google

STUDIES ON THE CONDITIONS OF ACTIVITY OF THE ENDOCRINE GLANDS

X. THE CARDIO-ACCELERATOR SUBSTANCE PRODUCED BY HEPATIC STIMULATION

W. B. CANNON AND F. R. GRIFFITH

From the Laboratories of Physiology in the Harvard Medical School

Received for publication February 1, 1922

In a previous paper of this series (1) evidence was presented that stimulation of the hepatic nerves causes a substance to appear in the blood passing through the liver that accelerates the denervated heart and induces a rise of blood pressure. We wished to secure information regarding the nature of this pressor and cardio-accelerator substance.

In the experiments to be described, as in the earlier series, we used cats under light, but complete, ether anesthesia. The hepatic nerves were stimulated by means of shielded electrodes which prevented any spread of current. The strength of stimulus was approximately constant in all cases, and the stimulation lasted uniformly 30 seconds.

FURTHER EVIDENCE THAT THE CARDIO-ACCELERATOR AGENT IS OF HEPATIC ORIGIN. The reason for attributing the faster heart rate to a substance produced by the liver was that excitation of the hepatic nerves induced the change when all the extrinsic nerves of the heart had been severed and the only means of transmission between the liver and the heart was supposed to be the blood stream. The connection of the liver with the heart through the inferior vena cava was not considered. Might it not be possible that a disturbance induced in the hepatic veins could be carried along the cava to the heart and the rate thus accelerated? This possibility we tested by stimulating the cava low in the thorax. No effect on the heart was produced and therefore that possibility was excluded.

Again, if a substance is produced by the liver we should be able to collect blood containing it and, on injecting that blood, we should expect to produce the usual results of hepatic stimulation. To carry out this experiment we have introduced a small catheter, oiled inside and out,

Digitized by Google

into the right jugular vein low in the neck and down past the heart into the inferior cava nearly to the diaphragm. The cava below the liver was then closed (the adrenals had previously been removed), and after the hepatic nerves had been stimulated for 25 of the standard 30 seconds, 10 cc. of blood were drawn through the catheter into a syringe. The experiment is difficult to perform, and we have had a number of failures. In one instance, however, we succeeded in securing 10 cc. of blood (at 12:33). The heart rate, which had previously risen 22 beats per minute in consequence of hepatic stimulation, rose 24 beats (from 182 to 206 beats per minute) in consequence of stimulating the nerves at the time of withdrawing the blood from the liver viens. At 12:34 the rate had fallen to 196. The cava below the liver, which had been opened after removal of the blood, was now closed again and (at 12:34) the blood was slowly, during 15 seconds, reinjected into the thoracic cava. The heart rate rose from 196 to 208 beats per minute; a minute later the rate had fallen to 192, and shortly thereafter it returned to 180. In a control test 10 cc. of blood were withdrawn (at 12:51), without stimulation of the hepatic nerves; its reintroduction (at 12:53) was not attended by an increase in the heart rate. As will be mentioned later, there is evidence that an extract of blood which has been clotted, or blood which is approaching coagulation, will accelerate the heart. The brief period during which the blood was withheld in this positive experiment (1 minute) and the failure of an effect when no stimulation occurred, though the blood was withheld for 2 minutes, seem to us to rule out the possibility that the increased rate was due to blood changes.

In the earlier study of the effect of hepatic stimulation on the heart rate it was noted that a greater cardio-accelerator action was commonly associated with digestion (1). This might be due to the influence of some material which, taken from the intestines into the blood and normally removed as the blood passes through the liver, is not removed if the hepatic nerves are stimulated. Thus the faster heart rate would be due to a failure of the liver, when stimulated through its nerves, to protect the heart against a cardio-accelerator agent produced in the alimentary canal. Stimulation might act by disturbing the circulation or by affecting the liver cells. The hepatic nerves, according to Opitz, constrict and lessen the blood flow in the hepatic artery much more than in the portal vein (2). Might it not be true that reduction of the arterial blood supply interferes with hepatic function so that material absorbed from the intestines is not removed from the portal blood? This possibility was tested by closing the hepatic artery in a well-fed, digesting

Digitized by Google

cat; the heart rate was not increased. For example, on January 20, at 11:10, hepatic nerve stimulation for 10 seconds increased the heart rate 18 beats per minute; at 11:15, the hepatic artery was closed without changing the rate a beat; nevertheless, stimulation of the hepatic plexus during the exclusion of arterial blood accelerated the heart 25 beats per minute. Furthermore, we found (Feb. 28) that when the portal vein was closed, hepatic nerve stimulation, which previously (at 3:32) had hastened the heart rate 24 beats per minute, still (at 3:42) raised the rate by 15 beats. And on April 6, after excision of the small and large intestines, the spleen and the pancreas, and ligature of the cava below the liver and of the aorta above the renal branches, hepatic stimulation still had as great an effect as before, speeding up the heart by 16 beats per minute. The absence of any change on closing the hepatic artery, and the continuance of the accelerator effect though the portal vein was closed or the alimentary canal removed, we interpret as showing that the accelerating agent is elaborated in the liver itself and does not come directly from the intestines.

The inference just drawn received further support in three cases cited in a previous paper (1, p. 362). In two of them, to which we can now add four more, acceleration on stimulating the hepatic nerves occurred though the animals, which had been well fed, were not digesting, and in a third, an animal which had been much excited, it failed to occur though the alimentary canal contained much meat in various stages of digestion. Thus hepatic stimulation may be quite effective in the absence of material coming from the intestine and may be ineffective when intestinal contents are present. In other words, the liver, not the intestine, is the source of the accelerating factor.

Feeding experiments. As was recalled above, the cardio-accelerator effect was observed in our previous study to be large in many of the animals which were actively digesting; but with a few exceptions (which we shall consider later) it was slight if the animals were fasting or in poor physical condition. This difference suggested testing the effects of different classes of food.

Carbohydrate feeding. As a rule, after potato has been fed shortly before the experiment or for several days previously, excitation of the hepatic nerves does not cause a faster heart rate than is observable when an animal has been without food for a day or two. The following results illustrate what we have noted:

February 28. Cat without food 2 days, fed potato 4 hours before operation. Stimulation of the hepatic nerves caused an increment of 8 beats per minute.

Digitized by Google

March 14. A repetition of the experiment of February 28; increment of only 4 beats.

March 17. Another similar experiment, with potato fed 3 hours previous to the operation. Increments of 6 and 8 beats.

March 28. Cat fed potato for 6 days preceding test. Hepatic stimulation caused increments of only 8 and 4 beats.

Stimulation of the hepatic nerves induces an increase of sugar in the blood (3). The foregoing experiments show that this effect does not influence the denervated heart. Other experiments, to be reported later, confirm this conclusion. The results of the foregoing tests do not indicate that carbohydrate feeding is favorable to a large cardiac acceleration of hepatic origin.

Fat feeding. Since the liver is supposed to desaturate the fatty acids and, by introducing double bonds, to prepare them for more ready metabolism (4), it might be assumed that the presence of plenty of fat in the blood would be favorable for the elaboration of material which would increase the heart rate. In our experience, however, feeding fat either continuously for several days or shortly before the experiment does not have an effect different from that observed in the fasting animal. For example, on March 21, an animal which had taken a meal of lard the night before and which was actively digesting it at the time of the experiment showed cardiac accelerations of only 8, 6 and 6 beats, respectively, on repeatedly stimulating the hepatic nerves. And on March 26, an animal which had been fed only lard for 5 days and was again fed on the morning of the experiment, so that it was actively digesting fat, showed no acceleration, or at most an increase of 1 beat per minute when the hepatic nerves were excited. Nothing in the evidence at hand points to fats being in any sense favorable to the development or to the efficacy of the hepatic agent that accelerates the heart.

Protein feeding. The figures recorded in the second column of table 1 in the previous communication on the effects of hepatic stimulation (1, p. 356) were derived from experiments in which the animals were digesting meat. The average acceleration of the heart in these instances was 15.5 beats per minute. The average acceleration in fasting animals, recorded in the first column of the table, was 5.2 beats per minute, an effect which, as just pointed out, corresponds to that seen after carbohydrate or fat had been fed. Our tests of protein feeding have yielded figures quite different from the negative results of feeding potato and lard. On April 5 a test was made on an animal which had

Digitized by Google

been fed for a week on all the lean meat it would eat and which had been given 100 grams of ground lean meat the previous afternoon. At the time of operation the intestines contained material undergoing digestion. Stimulation of the hepatic nerves caused increments of the heart rate as high as 12 and 14 beats per minute. On April 6 a similar experiment was performed with increments of 14 and 16 beats. After eating meat (3 hours before operation), on April 8, a cat showed accelerations of 26, 12 and 22 beats per minute. An animal given meat for 4 days before and also on the morning of the test (Nov. 22) had a heart rate faster by 13 and 14 beats after hepatic stimulation. The same stimulation (Dec. 22) in a cat digesting meat accelerated the rate in successive tests 32, 28, 20, 20 and 10 beats.

A modification of the experiments on protein feeding was tried in giving milk for some days and meat shortly before the operation. For example, on April 12, in an animal which had had milk for 3 days and which had eaten meat 4 hours previously, repeated stimulation of the hepatic nerves caused cardiac accelerations of 26, 12, 8 and 10 beats, respectively. And again, on April 14, in a similar experiment, the increases were 18, 16, 8, 10 and 12 beats. On the other hand, in two cases (April 13 and 16), milk-fed animals which were digesting meat, the maximal accelerations were 6 and 8 beats.

There have been five instances in which, though protein food was present in the alimentary canal and digestion had progressed, hepatic stimulation had very slight effects. In one of them, however, urethane was used as the anesthetic and perhaps determined the result—a possibility which we have not examined. In another, decerebration was tried, and when the hepatic nerves were stimulated, the blood pressure had fallen to 42 mm. Hg.—a level probably too low to permit the liver, which is sensitive to a deficient oxygen supply (1, p. 362), to respond. In the third the hepatic artery was found to have been for a long time closed. The fourth cat had been greatly excited before the experiment, especially while being anesthetized. In the fifth no possible explanation of the slight accelerations (4, 4 and 9 beats) can be given, unless it was that the animal had been fed liver instead of muscle—an improbable reason, we think.

From the foregoing evidence we draw the conclusion that as a rule the acceleration of the denervated heart on stimulating the nerves of the liver is more marked when the animal is digesting protein than when the animal has been fasting or subsisting on carbohydrate or fat.

Digitized by Google

THE EFFECT OF INJECTING AMINO ACIDS. The relation between protein feeding and the efficacy of hepatic stimulation, the fairly close chemical relation of adrenin to certain amino acids, and the demonstration by Barger and Dale that there are amines having sympathomimetic effects (5), suggested that the acceleration of the heart and the rise of blood pressure when the hepatic nerves are stimulated are due to a release of an agent, or agents, of this nature from the liver cells. It seemed possible that amino acids or amines, transported from the intestine to the liver, would there be removed from the portal stream by the cells and that when the cells are stimulated they would release these

TABLE 1

Effect of injecting mixed amino acids (except cystine) into small intestine

DATE	AMOUNT INJECTED	INCREASE OF HEART BEATS PER MINUTE FROM HEPATIC STIMULATION 30 SECONDS								REMARKS
		Before injection	Minutes after injection							
			5	10	15	20	25	30	40	
	cc.									
February 28	20	8			19	23		24		Fasting 48 hours
March 2	20	2	2	2	2	8		8	12	Fasting 36 hours
March 4	10	6, 4	10	10	6					Fasting 36 hours
March 7	10	6, 6	7	10	9	12	8	8		Fasting 24 hours
March 17	10	6, 8	8		14	10		8	2	Digesting potato
March 21	10	8, 6			2	10		2	6	Digesting fat
March 26	10	0	2.5			2		2		Digesting fat
March 8	10	8		10	6	6			8	Digesting potato
April 5	10	12, 14	10			10				Digesting meat

In the last four experiments the amino acid mixture was neutralized with sodium bicarbonate.

substances before having time to alter them or incorporate them. It was conceivable also that the faster heart rate was an expression of the specific dynamic action of certain amino acids in accelerating metabolism

The results of injecting into the small intestine a solution of mixed amino acids (except cystine) are given in table 1. The first experiment (Feb. 28) yielded such striking results that we thought we were about to make a big step forward. The second experiment was not wholly disappointing, but thereafter the results were so uncertain as to leave us no sound basis for a conclusion. As the table shows, the effects

Digitized by Google

(except in the first two experiments) were not notably different whether the introduction of the amino-acid solution was preceded by fasting or was accompanied by feeding carbohydrate, fat or meat. One might think that the amount injected was too small, especially in the later experiments; on one occasion, however, the night before the operation we gave by stomach tube 40 cc. of the solution to a cat which had been without food 48 hours, and introduced 25 cc. more in the morning shortly before anesthetizing, but we were unable by hepatic stimulation to accelerate the heart more than 6 beats per minute.

A variation on the foregoing experiments was made by use of the filtrate from a pancreatic digest of casein, containing presumably all the amino acids and some polypeptides. An injection of 0.1 cc. of this filtrate into the femoral vein increased the heart rate 10 beats per minute; a second injection, 1.0 cc., raised the rate from 178 to 208, i.e., 30 beats per minute. After 7 cc. of the digest had been injected into the small intestine, however, the heart rate was not altered to any noteworthy degree, nor was stimulation of the hepatic nerves made more effective. In another experiment the stimulating effect of the filtrate itself, when introduced intravenously was confirmed—1.0 cc. increasing the rate 20 beats per minute—but this effect, we must emphasize, should be distinguished sharply from an effect on the liver, such as appeared to be present in the experiment of February 28, table 1, and was revealed by a greater efficacy of hepatic stimulation.

On the supposition that the faster heart rate might in some manner be related to the specific dynamic action of protein, we have tested the effect of glycocoll. Lusk has reported that when it is given by mouth it increases the metabolic rate (6). We found, however, that an injection (during 10 minutes) of 10 cc. of 5 per cent glycocoll in mammalian Ringer into the femoral vein not only failed to increase the heart rate, but actually reduced it, and also reduced the influence of hepatic stimulation. A stimulation which, previous to the injection, had increased the rate 22 beats per minute had no effect immediately after the injection was completed and 6 minutes later caused an increment of only 6 beats. A second injection of the same amount during 16 minutes into a branch of the portal vein did not depress the rate to so great an extent, but, as before, it did not favor the action of the hepatic factor on the heart.

Alanine (1 and 2 cc., 5 per cent), cystine (1 cc., saturated solution), tryptophane (5 mgm. in 2.5 cc.), cystine (5 cc., 1 per cent), leucine (the same), asparagine (the same), glutamic acid (the same), aspartic acid

Digitized by Google

(5 cc., saturated solution), and phenyl-alanine (5 cc., 1 per cent), had either no effect or a negligible effect (a change of 2 beats) on the heart rate. Ammonia (1 cc., 1 per cent), introduced into the colon, if it acted at all, reduced the heart rate.

There is some evidence that tyrosine, closely related in chemical structure to adrenin, is capable of accelerating the heart. On April 14, injection of 2 cc. of a saturated solution in mammalian Ringer into the femoral vein caused an immediate rise of 5 beats (from 182 to 187) and within 3 minutes 5 beats more, to 192 per minute, where the rate remained for more than 10 minutes. Again on May 12, injection of 1 cc. of a saturated solution of tyrosine in mammalian Ringer increased the heart rate within 3 minutes by 15 beats per minute. Since tyrosine is more soluble in acid and alkaline solutions we tried such preparations, and with 2 mgm. were able to induce increases of 10 and 11 beats. But in no instance was there produced any greater effect of stimulating the hepatic nerves. Introduction in one case of 4 cc. of a 1 per cent tyrosine suspension into the colon, and in another case 2 cc. of the same suspension into the small intestine led to no change in the heart rate and to no increased action of the hepatic factor.

As is well known, tyramine in small amounts will raise blood pressure and in other ways act like adrenin. We found that when injected into a vein or into the intestine in small amounts it increased the heart rate remarkably but did not cause any considerable change in the efficacy of hepatic stimulation. Thus on November 8, after the introduction of 2 cc. of a 1 per cent solution of tyramine into the small intestine had raised the heart rate from 228 to 246 and later to 276 beats per minute, hepatic stimulation, which previously had increased the rate 8 beats, still increased it 8 and 5 beats. In order to induce a slighter acceleration of the heart rate from tyramine passing through the liver, and thus to permit, perhaps, a greater opportunity for action from any tyramine that might have been taken up by the liver cells and that might be released when they were stimulated, we tried introducing a smaller amount. On November 15, 10 cc. of a 0.002 per cent solution injected into the intestine raised the rate from 210 to 232 beats; the increment following excitation of the hepatic nerves, however, which previously had been 12 and 9 beats, continued thereafter 8 and 12 beats.

As stated in the first paragraph of this section, the concept which led us to undertake the observations on amino acids and amines was that these agents might be taken out of the portal blood by the liver cells during protein digestion and might be released when the nerves were

Digitized by Google

stimulated and thus influence the heart. In that case these substances should themselves act on the heart when introduced into the general circulation. This effect has been noted only in case of tryosine and tyramine. Furthermore, if our concept were correct, when these substances are introduced into the intestine there should be an increased effect on the heart from exciting the hepatic nerves. With the exception of the first two instances given in table 1, however, this effect has not been seen. It seems to us probable, therefore, that the hepatic agent is not an immediate product of digestion, but some material elaborated by the liver in the course of time.

EFFECT OF KNOWN OR SUPPOSED LIVER PRODUCTS ON THE DENERVATED HEART. Best known of the substances resulting from metabolic changes in the liver are glucose and urea. It has been claimed also that the liver produces and discharges catalase. Since our experiments led us to the conclusion that the accelerator agent is some substance elaborated by the liver cells, we decided to determine whether any of the known or supposed liver products could account for the effect on the heart of stimulating the hepatic nerves.

Glucose. Locke and Rosenheim noted that when the rabbit heart was perfused with oxygenated Ringer solution, the rate was considerably augmented by the addition of glucose to the solution (7). Stimulation of the hepatic plexus will cause a prompt liberation of glucose from the liver (3). It seemed possible, therefore, that the faster cardiac rate might be accounted for by a greater concentration of glucose in the blood going to the heart. The cardiac rates per minute at the times indicated and the increments of rate (in parenthesis) due to uniform repetitions of hepatic stimulation, before and after intravenous injection of glucose, are shown in the following records:

January 8. Cat's weight, 3.1 k. At 12.10, 1 cc. 5 per cent glucose injected during 13 seconds into femoral vein. No change in heart rate.

February 18. 11:44, 192 (6); 11:50, 189 (4); 12:23, 188 (1). At 12:27, 10 cc. 5 per cent glucose injected into femoral vein during 25 seconds. 12:32, 186 (10); 12:37, 188 (8). At 12:39, 20 cc. 5 per cent glucose injected. 12:44, 186 (10).

February 19. 11:23, 115 (7); 11:27, 124 (10). At 11:29, 10 cc. 5 per cent glucose injected into femoral vein. 11:31, 124 (12); 11:37, 128 (12); 11:44, 127 (11).

February 21. 11:24, 193 (9); 11:51, 209 (3). At 11:58, 10 cc. 5 per cent glucose injected into femoral vein. 12:02, 202 (6); 12:08, 206 (6); 12:15, 200 (2); 12:20, 200 (4).

March 24. Cat's weight, 3.5 k. 5:33, 198 (10); 5:40, 200 (6); 5:50, 196 (12). At 6:00, 5 cc. 5 per cent glucose injected into femoral vein. 6:03, 192 (8).

Digitized by Google

As the figures show, injection of glucose in an amount which would increase the content in the blood to a degree that might result from hepatic stimulation (e.g., March 24) failed to have any effect on the heart rate. And the injection of much larger amounts (10 or 20 cc. of a 5 per cent solution) in animals weighing about 3 kilos, likewise caused no noteworthy change. Furthermore, though the experiments of February 18 and 19 seemed to indicate that the glucose injections rendered hepatic stimulation more effective, the later experiments did not. We conclude, therefore, that the increments of heart rate due to stimulating the hepatic nerves are not the consequence of liberated glucose.

Urea. The liver is supposed to be the principal place for the formation of urea after absorption of amino acids from the intestine. Backman noted that when he perfused a rabbit heart with 2 per cent urea in oxygenated Locke's solution, the heart beat was increased in both amplitude and rate (8). Though there is no evidence that an output of urea from the liver can be induced by nervous stimulation, it seemed at least possible that discharged urea might account for the faster heart rate of hepatic origin. To test this possibility we injected urea into the femoral vein. The cardiac rates per minute at the times indicated and the increments of rate (in parenthesis) due to repeated stimulations of the hepatic nerves, uniform in strength and duration, before and after the injection of urea, are shown in the following records:

January 22. Cat, 3.7 k. 11:49, 204 (3); 11:54, 198 (0). At 12:12 injected into femoral vein during 10 seconds 2.25 grams urea in 4 cc. distilled water. Heart rate fell, rose and fell again, 194, 186, 184, 188, 200, 184, and at 12:24, 182 (0). At 12:29 injected during 10 seconds 2 grams urea in 2.5 cc. distilled water. Heart rate fell and rose, 182, 174, 176, 180.

April 13. Cat, 3.5 k. 3:17, 186 (6). At 3:23 injected into femoral vein during 2 minutes 10 cc. 5 per cent urea (0.5 gm.) in mammalian Ringer. No immediate change in heart rate. 3:26, 184; 3:30, 188 (8). Injected again, at 3:34, 10 cc. 5 per cent urea, but during 20 seconds. Heart rate dropped 18 beats per minute and quickly recovered. 3:35, 192; 3:38, 184; 3:41, 188 (0).

April 14. Cat, 3.1 k. 2:15, 188 (4); 2:23, 194 (4). At 2:33 injected into femoral vein during 70 seconds 10 cc. 5 per cent urea in warm mammalian Ringer. No change in heart rate, which was 188 beats per minute. 2:35, 188; 2:38, 185.

As revealed in these observations the effects seen by us in the cat, unlike those noted by Backman, show that urea has either no action on the rate of the isolated heart or a depressant action. Moreover, injection of urea solutions did not render hepatic stimulation any more efficacious in accelerating the heart.

Digitized by Google

Catalase. Burge has stated that, in consequence of hepatic stimulation, catalase is given off by the liver into the blood stream (9). He regards this as an agent for increasing the rate of oxidative processes in the body. Recent investigations have not shown any close relationship between the metabolic activity of organs, including the heart, and their catalase content (10). Granted, however, that Burge's claims are correct that nervous influences cause the liver to discharge catalase and that this ferment accelerates oxidation, the faster heart rate after hepatic stimulation might be explained. It seemed to us, therefore, that this possibility should be tested. We have tried the intravenous injection of catalase made from the liver, after the manner described by Battelli and Stern (11). The results were as follows:

February 5. At 11:50, 1 cc. catalase solution (1 cc. added to 10 cc. H_2O_2 produced in 10 minutes 25 cc. O_2, at 20°C. and 757.5 mm. Hg. pressure) injected into femoral vein; heart rate changed from 206 to 205 beats per minute. At 11:55, 2 cc. of the solution, change of cardiac rate, 204 to 206. At 12:03, 4 cc., rate, 202 to 203. At 12:20 both adrenal glands removed. At 12:22, 2 cc. catalase solution, rate 203 to 200. At 12:30, 4 cc., rate 196 to 182. At 12:38, 2 cc., rate 190 to 184. (Cardiac rate dropping after adrenalectomy.)

April 12. Cat, 3.5 k. At 12:11, 212 (26); 12:17, 212 (12); 12:25, 200, (8); 12:37, 188 (10). At 12:41, 5 cc. liver catalase in warm mammalian Ringer (1 cc. liberated 6 cc. O_2 from 20 cc. of 50 per cent H_2O_2 in 10 minutes) injected into femoral vein; heart rate changed from 186 to 184. 12:44, 184. At 12:47, 10 cc. catalase solution injected; heart rate unchanged, 184. 12:48, 188; 12:52, 188; 12:55, 180 (10).

The foregoing results give no support to the idea that catalase, increased in the blood stream, can cause acceleration of the cardiac rate. Even though hepatic stimulation may evoke a discharge of catalase from the liver, therefore, that would not explain the faster heart beat that occurs.

Bile. The failure to find any influence on the denervated heart of substances known or supposed to be given off by the liver into the blood stream led us to note the effect of injecting bile.

There was no encouragement in this experiment to lead us to make other tests.

November 22. Cat, 4.8 k. 2:24, 195 (13); 2:33, 194 (14); 2:36, 192 (4); 2:48, 188 (4); 2:55, 187 (0); 3:10, 188 (4). At 3:56. 0.75 cc. bile (taken from animal's gall bladder) injected into femoral vein during 8 seconds. Heart rate changed from 182 to 180 beats per minute. 3:59, 179; 4:01, 180. At 4:07, 1 cc. of bile injected with no change of heart rate, 184 before and 184 after.

Digitized by Google

THE EFFECT OF LIVER EXTRACTS. Since extracts of the adrenal medulla are capable of causing the changes produced by adrenal secretion, it seemed possible that likewise a substance might be extracted from the liver that would have the effects seen after stimulating the hepatic nerves. Our first attempts were directed toward obtaining an extract in Ringer solution. The liver was ground in sand and allowed to stand in the cold over night. In no cases did these simple extracts of the liver, thus delayed in being tested, induce any acceleration of the heart that was worthy of notice. We next turned our attention to the possibility of extracting by boiling in weak hydrochloric acid (0.4 per cent), and nearly neutralizing with sodium hydrate. The filtrate was then injected, with the following results:

January 19. Cat, 3 k. At 11:52, 2 cc. injected during 10 seconds; heart rate increased from 230 to 244 (14 beats per minute).

January 20. Same extract used January 19. At 12:03, 2 cc. injected in 11 seconds raised the heart rate 6 beats, from 234. At 12:07, 2 cc. of dissolved aqueous solution of the alcoholic precipitate of the extract was injected; rate unchanged. Later the same injection lowered the rate 6 beats. At 12:12, injection of 2 cc. of muscle extract, made by the method used in extracting the liver, increased the heart rate 6 beats, from 238. At 12:23, 3 cc. liver extract injected; rate increased 6 beats, from 240. Adrenals removed, 12:43. At 12:50, 3 cc. liver extract injected in 16 seconds; heart rate increased 6 beats, from 204.

January 21. Cat, 3.1 k., in poor condition. At 11:00, 2 cc. of the same extract used January 19, injected during 13 seconds, increased the heart rate 4 beats, from 176.

January 22. Cat, 3.7 k. At 10:22, 1 cc. of the extract used January 19, injected during 6 seconds, increased the rate 13 beats, from 171. A solution of the alcoholic precipitate of the extract had no effect; but the filtrate, evaporated and diluted with water, raised the rate 8 beats, from 174 (at 10:35). At 10:41, 1 cc. muscle extract, made as the liver extract was made, had the following effects on the heart beats in three repeated injections, +4, +1, −2. Thereupon, 1 cc. liver extract, at 11:35, accelerated the heart 15 beats per minute, from 175; and 3 cc., at 12:01, increased the rate by 39 beats.

January 25. Cat, 4.7 k. At 10:32, 1 cc. of the extract made January 19, raised the heart rate 26 beats, from 180. At 10:38, a fresh liver extract, made in the same manner January 24, raised the rate 28 beats from 178.

These results seemed very promising, and we thought that we had a substance which was peculiar to the liver in accelerating the pulse. It was unlike hepatic stimulation, however, in that it caused a fall of blood pressure instead of a rise. That was suspicious. In order to make sure whether liver extracts were peculiar in accelerating the denervated heart we made, on January 25, fresh extracts of liver, pancreas, intestinal mucosa, gastric mucosa, salivary gland and skeletal muscle.

Digitized by Google

In each case the tissue was minced, ground in sand, boiled in 5 parts of 0.4 per cent hydrochloric acid, nearly neutralized (while boiling) with sodium hydrate, and then filtered. Thus all the tissues were treated alike. When a uniform amount (1 cc.) of each of these extracts was injected (Jan. 26), the cardio-acceleration caused by each in succession was remarkably uniform. E.g., liver, 4 beats per minute; salivary gland, 5; gastric mucosa, 5; pancreas, 5; intestinal mucosa, 8; muscle, 4; liver again, 4. From these results we concluded that liver, extracted in the manner described, yielded no peculiar substance affecting the denervated heart. We were thus blocked from progress in that direction.

After these observations had been made we found that simple extracts of the liver in Ringer solution, if *fresh*, would accelerate the pulse. To obtain material we tied off with a strip of gauze the tip of one of the liver lobes and cut it away without any bleeding. It was then promptly weighed, ground in sand, mixed with the salt solution in the ratio of one part liver substance to five parts Ringer, and filtered through cotton. We found in our first test that 5 cc. increased the heart rate 6 beats per minute and caused also a rise of blood pressure. Extract of spleen made in the same manner increased the rate only one beat. Hepatic stimulation caused increments of 4, 6 and 9 beats. Here were effects of injection that corresponded closely with the effects of stimulation. Again the results seemed favorable to some explanation. On extending our tests and comparisons, however, we soon found that although extracts of spleen, intestinal mucosa and lymph gland, all made in the standard way, usually had less effect than liver extracts, a preparation of salivary gland was commonly most effective of all in speeding up the heart. E.g., on December 15, 5 cc. each of extracts of the following tissues (1 part in 5 of Ringer solution) increased the pulse rate as indicated: liver, 12 beats; spleen, 6; intestinal mucosa, 6; lymph gland, 8. On the other hand, when (Dec. 17) liver quickened the pulse 26 beats, and spleen 8, salivary gland quickened it by 44. Though this is true, stimulation of the chorda tympani nerve is not accompanied by any faster heart rate.

It may be that all these tissue extracts influence the heart by changing the blood. We have found that extracts of clotted blood prepared as were the extracts of tissues mentioned above produce cardiac acceleration, and further, that blood approaching coagulation has that effect. But in almost every instance, when the preparation of tissues or blood is injected, whether rapidly or slowly, the blood pressure is lowered.

Digitized by Google

This is different from hepatic stimulation. In a former paper (1, p. 359) reasons were given for regarding the rise of arterial pressure and the faster heart rate which result from hepatic stimulation, as both being due to an agent carried from the liver to the heart in the blood stream. If that reasoning was correct the agent must be different from these extracts—a peculiar substance, not only quickening the pulse but also raising pressure.

General considerations. We have hesitated to infer that the hepatic agent is a new internal secretion. In an earlier communication, presented when we had evidence that in some instances injection of amino acids into the intestines would render hepatic stimulation more effective (see p. 549), and when also we had noted the accelerator influence of tyrosine, we drew the tentative conclusion that the effects observed are probably not due to a true internal secretion produced by the liver, but to a discharge from its cells of amino acids, or amines, which are sympatho-mimetic in action (12). Further experimentation, however, as detailed above, has led us to the inference that the cardio-accelerator and pressor factor is not some digestive product let pass through the liver when the nerves are stimulated, or quickly freed from the hepatic cells under nervous stimulation, but that it is a material elaborated in the liver. A study of the action of known liver products does not permit us to attribute to any one of them the observed effects. After a survey of all the facts we do not at present see any other assumption to make than that a substance of special and unknown nature is discharged by the liver cells into the blood stream when the hepatic nerves are excited.

Unfortunately, our experiments have told us what the hepatic agent is not, but have given us almost no indication of what it is. In 1914, Berg (13) reported that in the liver cells of well-nourished animals (salamanders, rabbits) there are numerous small masses or droplets which are lacking in fasting animals, and that they appear when protein, but not when carbohydrate or fat, is fed. A positive test with Millon's reagent proved them to be protein in nature—differing from the protein of the cell in morphological characters and constituting a protein storage. In 1920, Stübel (14) confirmed Berg's results and found that this stored protein could be greatly reduced in the liver cells by injecting adrenalin subcutaneously. Since adrenalin mimics the action of sympathetic impulses and since the hepatic agent is liberated when the splanchnic sympathetics are stimulated, it is possible that the minute protein masses observed by Berg represent the stored cardio-accelerator

Digitized by Google

and pressor factor. In this connection the diminishing effect of repeated stimulation of the hepatic nerves in our experiments is of interest. This was a common, though not an invariable, observation. Examples of it are as follows: April 6, increments of 26–12–22–7–8–6 beats per minute; April 12, 26–12–8–10–10–8–8–4–8; April 14, 18–16–8–10–12–4–4; April 16, 8–0–1–3; May 6, with secondary coil 6 cm. out, 20–15–6–4–2, then with coil 4 cm. out, 16–10–2–4; November 22, 13–14–4–4–4; December 20, 10–11–7–6; December 22, 32–28–20–20–10. These reductions in the efficacy of hepatic stimulation could be explained on the assumption that the liver cells become gradually discharged of their storage of cardio-accelerator material and that they require more time than we gave them to elaborate it again.

Cannon and Mendenhall have noted that adrenalin injected into the circulation shortens the clotting time, if the blood is circulating through the abdominal organs but not if the flow is confined to the anterior part of the animal (15). Foster and Whipple have lately brought forward evidence that the liver is the actively productive source of fibrinogen in the body (16), and also that diets rich in animal protein favor a high level of blood fibrin as contrasted with fasting, or with diets of carbohydrate or fat. These observations suggest that the liver contains material favorable to blood clotting that can be brought out by stimulating the nerve supply of the organ. And it may be that this material or the blood altered by its presence induces a more rapid heart rate and a slightly higher arterial pressure. These are only indefinite hints, however, and further elaboration of them would be futile in the absence of pertinent observations.

SUMMARY

Stimulation of the inferior vena cava does not accelerate the denervated heart. The accelerator agent, appearing when the hepatic nerves are stimulated must, therefore, be conveyed in the blood stream.

The accelerator effect can be produced by reinjecting into the inferior vena cava blood drawn from the hepatic veins during stimulation.

The occurrence of a faster beat, though the hepatic artery or the portal vein is closed, proves that the acceleration is not due to failure of the liver, during stimulation, to protect the heart from an accelerator substance absorbed from the intestines.

Feeding carbohydrate or fat is without influence on the effectiveness of hepatic stimulation in evoking a faster beat of the denervated heart. As a rule the stimulation is most effective when meat or milk has been fed and the animal is digesting meat.

Digitized by Google

Intra-intestinal injection of mixed amino acids occasionally renders hepatic stimulation more effective, but the results are not constant.

Injection of glycocoll, alanine, cystine, tryptophane, cysteine, leucine, asparagine, glutamic acid, aspartic acid and phenyl-alanine into the femoral vein is without noteworthy effect on the heart rate. Tyrosine (saturated solution in mammalian Ringer) can increase the rate. But neither this intravenous injection nor introduction of tyrosine into the intestine causes hepatic stimulation to be more effective. The same is true of tyramine. The hepatic agent, therefore, appears to be not an immediate product of digestion, but some material elaborated by the liver in the course of time.

Of the known or supposed liver products neither glucose, nor urea, nor catalase, nor bile causes acceleration of the denervated heart.

Fresh liver extracts in mammalian Ringer solution or acid extracts accelerate the pulse, but so do similar extracts of other organs, and especially the salivary glands.

We conclude that a substance of special and unknown nature, which increases the rate of the denervated heart and raises blood pressure, is discharged into the blood stream when the hepatic nerves are stimulated. The possibility that this substance is related to the protein masses stored in liver cells (Berg) and discharged by adrenalin (Stübel) is considered.

BIBLIOGRAPHY

(1) Cannon and Uridil: This Journal, 1921, lviii, 353.
(2) Opitz: Quart. Journ. Exper. Physiol., 1912, iii, 303.
(3) See Macleod: Diabetes, New York, 1913, 72.
(4) Leathes and Mayer-Wedell: Journ. Physiol., 1909, xxxviii, p. xxxviii.
(5) Barger and Dale: Journ. Physiol., 1910, xli, 19.
(6) Lusk: Journ. Biol. Chem., 1913, xiii, 155.
(7) Locke and Rosenheim: Journ. Physiol., 1907, xxxvi, 205.
(8) Backman: Festschrift for Olof Hammarsten, 1906, 7.
(9) Burge: This Journal, 1917, xliv, 290.
(10) Seymore: Ibid., 1920, li, 525; Morgulis: Ibid., 1921, lvii, 125.
(11) Battelli and Stern: C. r. Soc. de Biol., 1904, lvii, 374.
(12) Cannon, Uridil and Griffith: Endocrinol., 1921, v, 730.
(13) Berg: Biochem. Zeitschr., 1914, lxi, 428.
(14) Stübel: Arch. f. d. gesammt. Physiol., 1920, clxxxv, 74.
(15) Cannon and Mendenhall: This Journal, 1914, xxxiv, 243.
(16) Foster and Whipple: Ibid., 1922, lviii, 429, 392.

Digitized by Google

VASOMOTOR RESPONSES OBTAINED BY SLOWLY INTERRUPTED FARADIC STIMULATION OF THE THORACIC SYMPATHETIC NERVE

LOYAL E. DAVIS

Contribution no. 94 from the Department of Anatomy, Northwestern University Medical School

Received for publication February 3, 1922

In a previous paper (1) it was pointed out that stimuli produced by a tetanizing current of sufficient intensity to elicit any vascular reflex from the central end of the cat's thoracic sympathetic nerve, divided just above the diaphragm and composed chiefly of fibers destined for the splanchnic nerve, resulted invariably in a rise of blood pressure. This is just the opposite of the effect obtained from central stimulation of the splanchnic nerve or thoracic sympathetic trunk in the dog. Auer and Meltzer (2) never obtained a rise in blood pressure from stimulation of the dog's splanchnic nerve, but rather drops amounting in some cases to as much as 60 mm. Hg. Burton-Opitz (3) also found that the characteristic reflex from stimulation of the central end of the dog's thoracic sympathetic nerve was a marked drop in blood pressure and he regards the splanchnic nerve as the depressor nerve for the abdominal viscera. It was also concluded that the visceral afferent pathway producing vasomotor reflexes probably consists of relays of short fibers with synapses in the gray matter of the spinal cord.

Obviously, the results obtained by stimulation of the thoracic sympathetic nerve with a tetanizing current immediately suggested the investigation of the use of a slowly interrupted faradic current upon this nerve. Hunt (4) first called attention to the fact that weak stimulation of an afferent somatic nerve elicited a fall in blood pressure. Gruber (5) and Gruber and Kretschmer (6), working upon the somatic nerves of cats anesthetized with urethane, showed that weak faradic stimuli with four interruptions per second, resulted in a drop in blood pressure in each instance, and that stronger stimuli with one interruption per second gave a depressor response in 93 per cent of the experiments. These results upon the somatic depressor reflex were verified by Vincent and Ogata (7).

Digitized by Google

Technique. Full-grown cats were used in these experiments to avoid variation in results due to age. The animals were anesthetized with urethane injected intraperitoneally. The dosage of the drug administered was 3 cc. per kilogram of body weight. Each 3 cc. of the solution contained 0.75 gram of the drug.

After isolating the brachial nerves in one extremity and preparing them for stimulation of their central ends, the carotid artery was prepared for blood pressure tracing and the vagi nerves divided. A tracheal cannula was then inserted and connected with an automatic compressed air machine which gave an interrupted supply of air at intervals corresponding to the normal respiratory rate.

To the cylinder which interrupted the air supply we attached a fiber wheel into which brass pegs had been driven at definite intervals upon its circumference. Each peg successively made contact with a spring to the end of which was attached a platinum needle. As each peg depressed the spring, the end of this platinum needle made contact with a cup of mercury. This entire apparatus was connected into the stimulating circuit. When the circuit key was closed a single stimulus would result each time contact was made into the cup of mercury. We so regulated the speed of the revolutions of the wheel that the stimuli occurred twice per second.

After mass ligation of the vessels in the chest wall, the thorax was opened upon one side parallel to the course of the seventh or eighth rib. The splanchnic nerve was then isolated just before it pierced the diaphragm, ligated and divided distally. The filament representing the thoracic sympathetic trunk below the point where the splanchnic is given off was cut, as were the rami communicantes of the tenth dorsal to the thirteenth dorsal segments inclusive. The splanchnic nerve and the lower portion of the thoracic sympathetic trunk, with which it is continuous, could then be raised by traction on the ligature at the time of stimulation. The usual precautions were taken to protect the nerve when we were not stimulating.

Results. The results obtained in the experiments upon ten cats were used as the basis for this article. Five other cats were used in this investigation but only upon these ten were depressor responses obtained from slowly interrupted stimulation of the brachial nerves and therefore these serve as a control of our results.

However, in all fifteen cats stimulation of the thoracic sympathetic nerve by a slowly interrupted current of sufficient strength to elicit any reaction resulted in a pressor response. As will be seen in figure

Digitized by Google

1, this response is similar to that obtained by the use of a tetanizing stimulus. This tracing also shows the typical depressor reaction resulting from stimulation of the brachial nerves with a slowly interrupted current of the same strength.

In none of the animals experimented upon was a drop in blood pressure obtained from slowly interrupted stimulation of the central end of the thoracic sympathetic nerve.

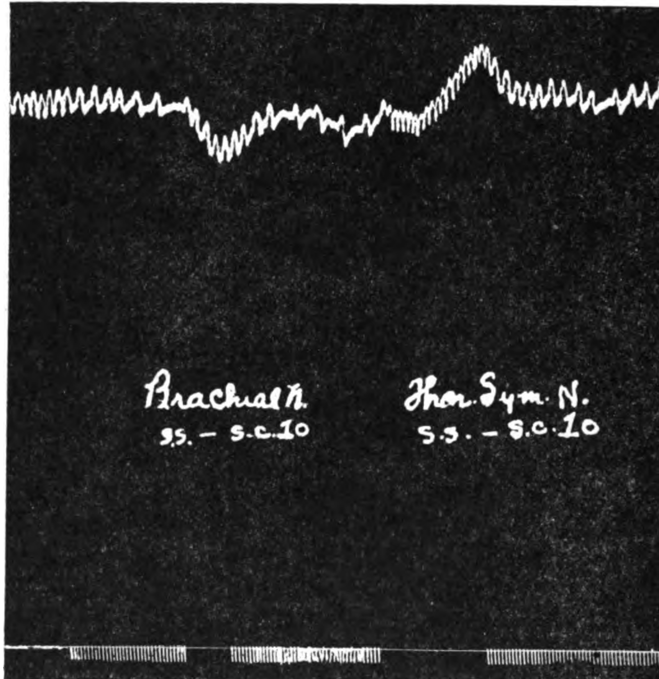


Fig. 1. Tracing showing the blood pressure response from stimulation of the central ends of the brachial and thoracic sympathetic nerves of the cat with a slowly interrupted faradic current.

Discussion. In the dog, stimulation of the central end of the vagus usually causes a pressor response, while in the cat the same stimulus causes a drop in blood pressure. On the other hand, the dog's splanchnic nerve gives a reflex resembling that obtained from the depressor nerve, while even with slowly interrupted stimuli the cat's splanchnic nerve is able to produce only a pressor reflex. This would seem to indicate that the connections which these nerves have with the vasomotor centers is radically different in these two mammals.

Digitized by Google

Whereas in the cat a somatic nerve may yield pressor or depressor reflexes under appropriate stimulation, the thoracic sympathetic nerve of this animal under these same conditions elicits only a pressor response. It will be recalled from the work of Ranson (8) that the somatic pressor path consists of short fibers in the tract of Lissauer and the apex of the posterior horn and that these chains of short neurons undoubtedly have many synapses. Very strong stimuli or rapidly repeated stimuli of moderate strength are necessary to break down the high synaptic resistance in the somatic pressor path. On the other hand we have shown that the visceral afferent pathway probably consists of relays of short fibers with many synapses in the gray matter of the spinal cord, yet those synapses are broken down by a slowly interrupted weak stimulus resulting in a pressor response.

CONCLUSIONS

1. Central stimulation of the lower end of the thoracic sympathetic nerve of the cat by a slowly interrupted faradic stimulus elicits a rise in blood pressure, although a similar stimulus of the brachial nerves causes a drop.
2. Stimulation of the central end of the dog's splanchnic nerve yields a depressor reaction while stimulation of the same nerve in the cat elicits a pressor response.
3. The synapses in the visceral afferent pathway of the spinal cord of the cat are easily broken down by slowly interrupted faradic stimuli of moderate or weak strength resulting in a pressor response.
4. Slowly interrupted faradic stimulation of the brachial nerves of the cat results in a depressor response which corroborates the work of Gruber.

BIBLIOGRAPHY

(1) Davis: This Journal, 1922, lix, 381.
(2) Auer and Meltzer: Zentralbl. f. Physiol., 1913, xxvi, 1316.
(3) Burton-Opitz: This Journal, 1916, xli, 103.
(4) Hunt: Journ. Physiol., 1895, xviii, 381.
(5) Gruber: This Journal, 1917, clii, 214.
(6) Gruber and Kretschmer: This Journal, 1918, clvi, 222.
(7) Ranson and von Hess: This Journal, 1916, xxxviii, 128.

Digitized by Google

THE ISOLATION OF A SUBSTANCE FROM URINE HAVING PROPERTIES OF CITRIC ACID: DESCRIPTION OF AN APPARATUS FACILITATING THE WORKING WITH SMALL VOLUMES OF GAS

SAMUEL AMBERG AND MARY E. MAYER

From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago, and The Mayo Foundation, University of Minnesota, Rochester, Minnesota

Received for publication February 10, 1922

The normal urine of human beings yields pentabromacetone when treated by the method used by Kunz (11) for the determination of citric acid in wines. Briefly the method is: Potassium bromid and sulfuric acid are added to urine and the mixture heated to just below 55°C. A solution of potassium permanganate is added slowly. When the reaction is completed the solution is cleared with ferrous sulfate. A crystalline precipitate of pentabromacetone is deposited. The method is fairly accurate for quantitative determinations of citric acid, if a correction is applied. Amberg and McClure (2) found that if a volume of 50 cc. is used it is necessary to add about 5.5 mgm. citric acid ($C_6H_8O_7.H_2O$) to the end result. Urine also gave a positive test when subjected to the treatment used by Salant and Wise (14) for the demonstration of citric acid in urine. This treatment makes use of the Denigès reaction (6), (7). A solution of citric acid is heated after the addition of a solution of mercuric sulfate in sulfuric acid. The addition of potassium permanganate drop by drop to the hot solution causes the formation of a white precipitate. Acetonedicarboxylic acid, formed by the action of the potassium permanganate on citric acid, in the presence of nascent bromine is transformed to pentabromacetone while in the presence of mercuric sulfate it forms an insoluble mercury compound. The results of Amberg and McClure indicated the presence of a substance in urine which readily yields acetonedicarboxylic acid. None of the substances hitherto known to occur in urine had been shown to possess this property. Because acetonedicarboxylic acid is a characteristic product of citric acid cleavage and because citric acid, a normal constituent of milk of the human being, is not foreign to

Digitized by Google

the organism, it was concluded that the acetonedicarboxylic acid was derived from citric acid. The proof was not absolutely unquestionable and the present study was undertaken to substantiate further the conclusion by the isolation of citric acid with the formation of characteristic salts and by elementary analyses. In this we were not successful, but the results of our experiments support the conclusion that citric acid is a constituent of the urine of normal human beings. After a number of preliminary experiments the following procedure for the attempted isolation of citric acid was adopted.

If the urine is strongly acid its acidity is reduced to weakly acid reaction (litmus) with sodium hydroxid. Lead acetate is added to slight excess and then ammonium hydroxid to strongly alkaline reaction. The mixture is repeatedly stirred for about 12 hours when the precipitate is filtered off and washed by being taken up in water alkaline with ammonium hydroxid. The filtrates are evaporated over a free flame to convenient volume; the reaction becomes acid during evaporation. The solution is freed from lead by treatment with hydrogen sulfid and the filtrate and washwater from the lead sulfid is evaporated to a syrup, with loss of the excess hydrogen sulfid. Some crystalline material separates which is filtered off and freed from adhering mother liquor by washing with ice-cold water. The precipitate is discarded. Barium acetate (30 per cent) is added to the thin syrupy solution until no more precipitate is formed. Sometimes barium hydroxid was added to weakly alkaline reaction, but this was not found to be of special advantage. After the addition of 95 per cent alcohol, two to three times the volume, the precipitate is allowed to settle. The supernatant fluid is syphoned off, the precipitate is repeatedly washed with 50 per cent alcohol, and is finally collected on a filter. The precipitate is extracted two or three times with hot water acidified with ortho phosphoric acid, and filtered off after each extraction. The filtrates are evaporated to a small volume and treated with moderate excess of sulfuric acid. Plaster of Paris is added, and the evaporation continued to dryness. The residue is divided as finely as possible with a spatula and extracted with ether in a Soxhlet apparatus, in which the ether is renewed several times. The ether extract is evaporated and the residue taken up in water and filtered from some sticky material which is discarded. The filtrate contains phosphoric and sulfuric acids. Therefore, lead carbonate is added in as slight excess as possible, the liberated carbon dioxid is boiled out and ammonia is added to make the solution about 2 per cent. The mixture is left

Digitized by Google

standing a day or more, repeated shaking being necessary, or it is heated with ammonia, filtered, and the precipitate taken up several times in ammoniacal water and filtered. On evaporation the solution becomes acid, and it is free from phosphoric and sulfuric acids. The lead is removed with hydrogen sulfid and the filtrate from the lead sulfid is evaporated to dryness. The residue is redissolved in water and filtered from a small amount of undissolved material. The color of this solution is usually light yellow; sometimes it is rather highly colored. If this is the case, it is heated with a small amount of animal charcoal. This is to be avoided if possible, since animal charcoal absorbs citric acid. The strongly acid solution is neutralized approximately with sodium hydroxid and treated with barium acetate solution until no more precipitate forms. The precipitate is collected and washed with graded dilutions of alcohol. This precipitate is the crude product.

From an accumulation of urine 6.8 grams of this crude product were obtained. Calculated from the amount of pentabromacetone obtained from part of it, it contained at least the equivalent of 1.6 grams of citric acid; that is, about one-half of it may have been barium citrate.

For further purification the crude product is again extracted with water acidified with phosphoric acid. The filtrates are evaporated to a small volume and 5 per cent solution of mercuric sulfate in sulfuric acid is added until no more precipitate is formed. The precipitate is discarded. Mercury is removed from the filtrate by treatment with hydrogen sulfid, excess of which is removed by boiling, the mercuric sulfid is filtered off and the filtrate is treated with lead carbonate as before. On evaporation, the ammoniacal filtrate becomes acid and a small amount of white precipitate separates. This is collected separately (A), and separated from the filtrate (B). (A) is washed well with water, taken up in water, and treated with hydrogen sulfid. The filtrate is freed from hydrogen sulfid by evaporation and the residue is taken up in water. On the addition of barium chlorid to the acid solution, some precipitate forms. Barium chlorid is added very carefully until the solution does not give any further precipitate, nor react with sulfuric acid. The precipitate is filtered off, the filtrate evaporated to dryness and the residue extracted with alcohol. On evaporation the alcoholic solution becomes a syrup which leaves very little residue when taken up in water; the residue is removed by filtration. To the strongly acid solution barium acetate is added. A snow-white precipitate forms, dissolves, and reforms on addition of more barium acetate, as happens on addition of barium acetate to citric acid.

Digitized by Google

Dried in a desiccator over sulfuric acid this white precipitate obtained from the crude product weighed 101.8 mgm. On drying at 163°C. it lost 7 mgm. of water. Ninety-four and eight-tenths milligrams of the dry substance yielded on analysis 79.5 mgm. barium sulfate which corresponds to 89.7 mgm. of dry barium citrate and it gave an equivalent amount of pentabromacetone.

The bulk of the filtrate (B) from the lead carbonate is evaporated further and more precipitate forms. The lead is removed from the precipitate suspended in the solution and the filtrate is evaporated to a small volume. A solution of barium chlorid carefully is added until no more precipitate forms. The filtrate from this precipitate is evaporated to dryness, taken up in water, filtered from a small residue and precipitated with barium acetate. After the precipitate was washed with water, dilute alcohol and strong alcohol, it weighed approximately 0.6 gram.

There is scarcely a step in the procedure which does not entail some loss, a conclusion drawn from the positive Denigès' reactions of the filtrates and washings. The substance used for the analyses was not quite pure, but we decided to use it rather than to lose it all by further efforts at purification.

It is known that citric acid heated with strong sulfuric acid gives off carbon monoxid, molecule for molecule. If the heating is done in a current of carbon dioxid, the carbon monoxid can be collected over potassium hydroxid permitting a quantitative determination of citric acid (16). If a substance gives pentabromacetone and carbon monoxid in equivalent amounts, it may be assumed that this substance is citric acid. The carbon monoxid determination was carried out as follows:

The dry substance is placed in a small Erlenmeyer flask and is moistened with water. The flask is closed with a three-hole rubber stopper, connected by one glass tube to the carbon dioxid generator, and by another to a gasometer charged with 50 per cent potassium hydroxid. From a small separatory funnel, inserted through the stopper, concentrated sulfuric acid is dropped into the flask which is then heated on a water bath over an asbestos plate; the liberated gases pass into the gasometer with the current of carbon dioxid. One to two hours after the collection of the gas, the volume is read; the gas is then transferred to an absorber filled with ammoniacal cuprous chlorid solution, and the carbon monoxid absorbed. The gas remaining is transferred to a measuring apparatus filled with water, in order to determine the necessary correction.

Digitized by Google

For our purposes an apparatus was devised to facilitate the handling of small volumes of gas without loss (fig. 1). The gas liberated in the reaction is collected in a nitrometer over 50 per cent potassium hydroxid. When the gas is to be transferred to the absorber, the capillary glass tube adjoining the stopcock of the nitrometer is filled with water. A short gasometer is fitted to this glass tube with a rubber stopper. Leading off from a small trough at the bottom of this gasometer is a side tube to which the rubber tube of a levelling bulb is attached. This gasometer is filled with ammoniacal cuprous chlorid solution from the levelling bulb, which is then lowered. The gas is transferred to the absorbing gasometer with the levelling bulb of the potassium hydroxid gasometer. The apparatus is sufficiently elastic to permit considerable shaking. When the absorption is complete a second short gasometer filled with water can be attached, and any remaining gas can be transferred and the volume read. The apparatus could easily be modified to meet special demands.

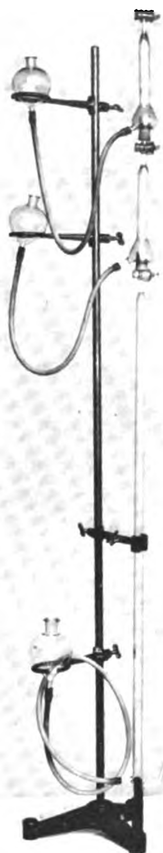


Fig. 1. Apparatus devised for the determinations of small amounts of carbon monoxid.

For the determination of carbon monoxid 0.1736 gram of the air dry barium precipitate was used, giving 0.0107 gram of carbon monoxid, an amount equivalent to 80.3 mgm. of citric acid (calculated $C_6H_8O_7.H_2O$). In determinations of pentabromacetone the Gooch crucibles containing the pentabromacetone precipitates were weighed after drying 24 hours in a desiccator over concentrated sulfuric acid. The pentabromacetone was washed from the crucibles with acetone, the crucible was dried and weighed again and thus possible errors from acetone insoluble impurities were avoided. For this determination 0.143 gram of the purified substance was taken; the yield of pentabromacetone was equivalent to 65.3 mgm. of citric acid.

Digitized by Google

It has been stated that the barium precipitate does not represent pure barium citrate. Before preparing the pentabromacetone the barium was precipitated and 111.6 mgm. barium sulfate were obtained. This amount is equivalent to 136 mgm. barium citrate, with 3½ molecules water of crystallization. The pentabromacetone obtained corresponds to 132.6 mgm. of barium citrate, while 143 mgm. of the barium precipitate was used. The carbon monoxid is equivalent to 163.1 mgm. barium citrate, while 173.6 mgm. of the barium precipitate was used. The amount of citric acid which should be present in 173.6 mgm. of the barium precipitate calculated from the amount of pentabromacetone found in 143 mgm. of the substance is 79.3 mgm., while with the carbon monoxid method 80.3 mgm. were found. The remaining part of the barium precipitate was dried at 164°C.; 119 mgm. yielded 8.16 mgm. carbon monoxid, an amount equivalent to 61.2 mgm. of citric acid; 127 mgm. yielded an amount of pentabromacetone equivalent to 63.2 mgm. citric acid. The amount of citric acid to be expected in 119 mgm. of material is 59.3 mgm. calculated from the pentabromacetone obtained from 127 mgm., while with the carbon monoxid method 61.2 mgm. were found.

Some test experiments with barium citrate dried at 164°C. are tabulated:

	BARIUM CITRATE	CITRIC ACID	
		Calculated	Determined
	mgm.	*mgm.*	*mgm.*
Pentabromacetone method	177.6	94.4	93.9
	191.5	101.8	101.5
	170.7	90.8	89.7
Carbon monoxid method	131.3	69.8	70.4
	109.8	58.4	58.2

The results obtained with the material isolated from urine show a rather good agreement between the amounts calculated and those found, taking into consideration that the pentabromacetone method may give values a little low and the carbon monoxid method values a little high. It is questionable whether the result of the second determination, not so good as that of the first, is owing to errors of method entirely. Our substance was not quite pure, and while pure barium citrate does not change when water of crystallization is driven off, an

Digitized by Google

impurity in our material may have undergone some change; which might account for the discrepancy in the results.

Our material was sufficiently free from organic impurities that on heating with concentrated sulfuric acid the color remained pale yellow, as in control experiments with citric acid or citrates. With material less pure the fluid becomes dark or even black.[1]

The results were obtained with material from one accumulation of urine; not enough material was obtained from other accumulations for similar analyses, because efforts at purification resulted in loss.

Only a few efforts were made to isolate our substance in the form of a calcium salt. It was very difficult to obtain any crystalline precipitates and if such occurred they were insignificant in amount. The difficulties here are as great as those encountered in efforts to isolate lactic acid from urine as a zinc salt. When calcium carbonate was added in place of barium acetate and the carbon dioxid was removed by heat, the neutral filtrate became acid and a small amount of a heavy crystalline precipitate separated rather early in the evaporation. This precipitate gave pentabromacetone and a positive Denigès' reaction. In one instance about 90 mgm. of this precipitate was obtained.[2]

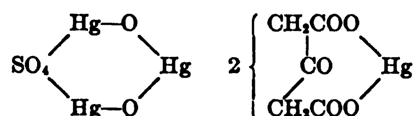
With a small amount of good material the reaction of Sabanin-Laskowsky was carried out. The barium was carefully removed with sulfuric acid; a slight excess of ammonia was added to the filtrate which was evaporated. The dry residue was taken up in strong ammonia. The solution was placed in a sealed tube and heated to 134°C. for six hours as recommended by Scheibe (15). After cooling the solution was taken from the tube and left standing in the air, whereupon a blue color developed. The reaction was positive. Henkel says that this reaction is given by citric and aconitic acids but the latter does not yield pentabromacetone under the conditions of our experiments.

[1] The impurities included in such specimens may also give rise to carbon monoxid, and account for the discrepancy between results of the two methods. For instance, a specimen dried at 164°C. weighing 141.5 mgm. gave pentabromacetone corresponding to 54 mgm. citric acid or 101.7 mgm. of dry barium citrate. The carbon monoxid method indicated 87 mgm. citric acid in 159.2 mgm. of this specimen, while the calculation, from the pentabromacetone determination gave 60.8 mgm. of citric acid. The mixture became black when some of the substance was heated with sulfuric acid.

[2] The fact that this precipitate formed at acid reaction made us think of dicalcium citrate as obtained by Henkel (8) from milk, but the specimen dried at 100°C. gave 27.2 per cent calcium oxid on burning; dicalcium citrate yields 24.4 per cent, and tricalcium citrate 32.55 per cent.

Digitized by Google

Several substances such as aconitic, itaconic and citraconic acids yield a positive Denigès' reaction, although they do not behave exactly as citric acid does in the course of the reaction. It is not certain that precipitates from these acids contain acetonedicarboxylic acid, particularly, since they do not yield pentabromacetone. Nevertheless, precipitates obtained from our substance by the method of Denigès, treated with hydrogen sulfid, and extracted with ether, on evaporation of the ether left a dry residue which in water solution gave a purple color with ferric chlorid and a positive Legal reaction for acetone. The ferric chlorid reaction could not be obtained if the watery solution had been boiled first; by boiling, acetonedicarboxylic acid is decomposed to carbon dioxid and acetone. Some efforts were made to isolate pure acetonedicarboxylic acid from such precipitates. Denigès reported that it is possible to obtain pure acetonedicarboxylic acid from citric acid by way of the mercury compound. The proof of the purity of this acetonedicarboxylic acid rested on its mercury content.[3] A mercury compound made with acetonedicarboxylic acid prepared by the method of Pechmann contained the same amount of mercury. The formula as signed by Denigès to the mercury compound of acetonedicarboxylic acid is:



It is very doubtful whether the mercury compound of acetonedicarboxylic acid is of uniform composition. Salant and Wise (14) found it necessary to construct a table based on analytical results which gives the weight of the mercury compound obtained from different amounts of sodium citrate under definite conditions. With the aid of this table and within its limits, quantitative determinations of citric acid in aqueous solutions could be made. On repeating the experiment of Denigès we were able to obtain crystalline residues from the ether extract which gave the reactions described by Denigès, but the melting point of these residues varied from about 117 to 125°C. and were associated with decomposition. Denigès did not give the melting

[3] These precipitates when dissolved in dilute hydrochloric acid, and shaken with ether give up to the ether a mercury compound. This property may perhaps be used for purification.

Digitized by Google

point of acetonedicarboxylic acid prepared by him, and Pechmann (13) gives for the pure acid a melting point of 135°C. with decomposition.[4]

The mercury compound obtained from our substance gave precipitates the consistency and color of which varied from yellow and waxy to white and crystalline. Some of the crystalline precipitates contained white needles, and the melting point varied from 117 to 120°C. with decomposition. There was a great similarity between the properties of the ether residues obtained from our substance and those obtained from citric acid.

The work of Amberg and McClure (2) was undertaken in order to study the fate of citric acid introduced into the organism under various conditions. It was expected that some information would be gained concerning oxidative processes and their disturbances in the organism, but it was not expected that information concerning the oxidative power of organisms in general would be obtained. Dakin (4) very justly says, "The specific character of animal oxidations is most remarkable especially when phenomena such as those presented by diabetes and alcaptonuria are concerned. In these conditions oxidation of a readily oxidizable product of metabolism (glucose, homogentisic acid) may be completely restrained without impairing in the least the capacity of the body for effecting the oxidation of other substances." Batelli and Stern (3) working with animal tissues have shown that the oxidation of various organic acids proceeds under various conditions. Dakin's very interesting review of physiologic oxidations (5) makes the conclusion nearly unavoidable that the organism can bring about oxidations by various mechanisms. The excretion of readily oxidizable substances is of great significance in the light of such considerations, whether these are introduced from without or formed in the organism as an intermediary product of metabolism. The total amount excreted is of importance but of no less importance are the variations in the excretion of the individual acids. It may be suggested that a search for alcohols and aldehydes should be productive of results in certain conditions.

When salts of the lower aliphatic acids are present in sufficient concentrations they may exercise an influence on numerous biologic reactions. Among these are chemotaxis (Wolf, 17), phagocytosis (McJunkin, 12), and enzyme action (Amberg and Loevenhart, 1). Jobling, Eggstein and Petersen (19) showed that citrates particularly have an accelerating influence on tissue and serum esterase.

[4] Crude acetonedicarboxylic acid is readily prepared by the method of Pechmann, by the modification by Koessler and Hankel (10), or by the method of Denigès, but the purification is rather difficult.

Digitized by Google

In such reactions the individual acid exercises a more or less specific function:

In summarizing it may be said that the substance isolated from urine in the form of an impure barium compound had the following properties in common with citric acid.

1. It gave the pentabromacetone and the typical Denigès reactions.
2. It yielded pentabromacetone and carbon monoxid in amounts closely equivalent.
3. It did not char on heating with concentrated sulfuric acid.
4. It gave the Sabanin-Laskowsky reaction.
5. Precipitates obtained according to Denigès' method behaved very similarly to precipitates from citric acid. From these precipitates a dry ether residue could be obtained, the aqueous solution of which gave the characteristic color reaction for acetonedicarboxylic acid with ferric chlorid.

The results support our contention that citric acid occurs in the urine of normal human beings, but still, they do not convey absolute proof, which rests solely on the isolation of the acid or its salts in a form pure enough for identification.

BIBLIOGRAPHY

(1) Amberg and Loevenhart: Journ. Biol. Chem., 1908, iv, 149.
(2) Amberg and McClure: This Journal, 1917, xliv, 453.
(3) Battelli and Stern: Biochem. Zeitschr., 1911, xxxi, 478.
(4) Dakin: Oxidations and reductions in the animal body, New York, 1912.
(5) Dakin: Physiol. Rev., 1921, i, 394.
(6) Denigès: Compt. rend. l'Acad. d. Sci., 1899, cxxviii, 680.
(7) Denigès: Compt. rend. l'Acad. d. Sci., 1901, cxxxi, 32.
(8) Henkel: Landwirtschftl. Versuchs-Stationen, 1891, xxxix, 143.
(9) Jobling, Eggstein and Petersen: Journ. Exper. Med., 1915, xxii, 701.
(10) Koessler and Hanke: Journ. Amer. Chem. Soc., 1918, xl, 1716.
(11) Kunz: Arch. f. Chem. u. Mikrosp., 1914, vii, 285.
(12) McJunkin: Arch. Int. Med., 1918, xxi, 59.
(13) Pechmann: Liebig's Annalen, 1891, cclxi, 151.
(14) Salant and Wise: Journ. Biol. Chem., 1916–17, xxviii, 27.
(15) Scheibe: Landwirtschftl. Versuchs-Stationen, 1891, xxxix, 153.
(16) Spica: Chem. Ztg., 1910, xxxiv, 1141; Chem. Abstr., 1911, v, 541.
(17) Wolf: Journ. Exper. Med., 1921, xxxiv, 375.

Digitized by Google

THE INFLUENCE OF MEAT UPON PHYSICAL EFFICIENCY

S. H. BASSETT, EVELYN HOLT AND F. O. SANTOS

From the Physiological Laboratory, Cornell University Medical College, New York City

Received for publication February 17, 1922

The mystery regarding the significance of meat in the dietary is yet to be clarified. In the following experiments there were four different periods of one week in length. During the first week the usual normal diet was taken by each individual. During the second week a luncheon containing 300 grams of beef was served, with bread, butter and boiled potatoes, in the laboratory. This quantity of meat is the amount which was contained in the daily war ration of the French and Italian soldier. During the third week little or no meat was taken, and during the fourth week the same procedure was followed as in the second week. The four individuals engaged in this work analyzed their urines daily for nitrogen; all wore pedometers for measuring the number of their daily movements. They also were instructed to walk a given distance both morning and late afternoon on their way to and from the college, striving to accomplish the walk in the shortest time possible. On completing the walk the pulse was counted and then the time noted until the pulse rate became normal once more. This has been suggested by the work of Lewis, Cotton and Rapport (1) as a test of physical fitness.

The subjects were an instructor in the laboratory (R.), a Philippino (S.) working for a higher degree, a man (B.) and a woman (H.) student. The first named was in perfect physical condition after a summer in the Maine woods; the fourth was fond of long walks and tennis in which she indulged during the experimental periods. The other two subjects were in good general health. The basal metabolism of S., who weighed 40 kgm., was 41 calories per square meter of surface, the normal value.

The observations may be thus summarized:

Digitized by Google

TABLE 1

Subject B., weight 64 kgm.

	FIRST WEEK		SECOND WEEK		THIRD WEEK		FOURTH WEEK	
	Ordinary diet		High protein		Low protein		High protein	
Average number of steps per day	14,253		12,636		14,840		13,616	
Urine N (average) grams	13.14		17.44		8.68		18.34	
Maximum-minimum	14.08–11.31		18.57–17.12		11.37–7.53		21.67–15.48	
	a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk	11.43	12.4	12.7	14.5	14.0	17.0	16.5	15.0
Time in minutes to make 1000 steps	8.51	8.50	8.80	9.70	9.33	9.50	9.45	9.38
Pulse:								
Before exercise	71.0	71.0	73.0	75.0	73.0	74.0	77.0	79.0
End of exercise	86.0	85.0	102.0	101.0	100.0	93.0	107.0	102.0
After 3 minutes					87.0	80.0	91.0	88.0
After 5 minutes	72.0	75.0	86.0	79.0	84.0	77.0	88.0	84.0

TABLE 2

Subject H., weight 58 kgm.

	FIRST WEEK		SECOND WEEK		THIRD WEEK		FOURTH WEEK	
	Ordinary diet		High protein		Low protein		High protein	
Average number of steps per day	21,520		21,503		19,691		20,880	
Urine N (average) grams	9.64		14.72		8.02		16.91	
Maximum-minimum	10.43–8.80		17.06–13.34		8.78–6.86		19.38–15.83	
	a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk	26.0	27.0	26.0	27.0	26.0	27.5	26.0	27.5
Time in minutes to make 1000 steps	7.0	7.3	7.0	7.3	7.0	7.4	7.0	7.4
Pulse:								
End of exercise	112.0	107.0	120.0	105.0	121.0	117.0	113.0	114.0
After 4 minutes	78.0	81.0	81.0	83.0	82.0	82.0	81.0	78.0
After 5 minutes		74.0		81.0		75.0		75.0

Digitized by Google

TABLE 3

Subject R., weight, 58 kgm.

	FIRST WEEK	SECOND WEEK	THIRD WEEK		FOURTH WEEK	
	Ordinary diet	High protein	Low protein		High protein	
Average number of steps per day................	14,980	13,145	16,415		14,965	
Urine N (average) grams..	12.5	18.9	12.3		18.9	
Maximum-minimum......	14.9–10.1	19.6–18.0	14.3–10.1		19.7–17.6	
	a. m.	*a. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk..................	20.9	22.0	21.8	19.1	21.9	19.3
Time in minutes to make 1000 steps..............	9.03	9.14	9.04	9.48	9.07	9.75
Pulse:						
Start of exercise..........	70.0	69.0	69.0	72.0	70.0	71.0
End of exercise...........	96.4	96.0	91.0	95.0	94.0	101.0
Time in minutes of return to normal.........	1.17	1.25	1.37	1.82	1.55	2.05

TABLE 4

Subject S., weight 40 kgm.

	FIRST WEEK		SECOND WEEK		THIRD WEEK		FOURTH WEEK	
	Ordinary diet		High protein		Low protein		High protein	
Average number of steps per day...............	13,902		14,552		15,695		14,808	
Urine N (average) grams	12.36		15.78		7.89		15.77	
Maximum-minimum.....	14.07–9.34		18.65–13.44		8.99–7.31		16.88–13.15	
	a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
Time in minutes of test walk..................	28.2	28.5	28.0	28.5	27.3	27.8	27.4	27.5
Time in minutes to make 1000 steps.............	9.24	9.34	9.17	9.34	8.99	9.18	9.04	9.13
Pulse:								
Before exercise..........	85.0	85.0	84.0	85.0	80.0	81.0	80.0	81.0
End of minute..........	111.0	113.0	114.0	110.0	120.0	112.0	121.0	119.0
After 3 minutes.........	97.0	97.0	94.0	98.0	85.0	88.0	79.0	88.0
After 5 minutes.........	90.0	95.0	87.0	90.0		80.0		80.0

Digitized by Google

SUMMARY

The results show that the presence or absence of meat from the dietary, during periods as long as one week, has no demonstrable effect upon the capacity of doing an amount of work so graded as to reach the limit of the physical capacity during a short period of time. This accords with the doctrine of Chittenden (2). There was a distinct and uniformly present sense of sleepiness for 2 or 3 hours during the afternoon period following the ingestion of 300 grams of meat. Removal of meat from the dietary for a period of one week did not diminish the sense of well-being in the individuals investigated. The well-nigh universal opinion that meat ingestion is important for the maintenance of physical strength is not to be disregarded, but the experimental evidence in favor of this conception has yet to be produced.

BIBLIOGRAPHY

(1) Lewis: Medical Research Committee, Special Rep., Series no. 8, London, 1917.

(2) Chittenden: Physiological economy in nutrition, New York, 1904.

Digitized by Google

THE GASTRIN THEORY PUT TO PHYSIOLOGICAL TEST[1]

A. C. IVY AND J. E. WHITLOW

From the Department of Physiology, Loyola University School of Medicine

Received for publication February 27, 1921

In 1906 Edkins (1) discovered that 0.4 per cent HCl extracts of pyloric mucous membrane when injected intravenously caused a secretion of gastric juice. This observation with certain qualifications has been confirmed by every investigator who has repeated the procedure and is without question a fact. With the exception of the cardiac mucous membranes, Edkins believed this to be an action specific for the pyloric mucous membrane and he termed the active substance of the extract "gastric secretin." Gross (2) in the same year offered evidence that led him to believe that the mucosa of the pyloric portion of the stomach elaborated a "gastric hormone" when food substances were brought into contact with it. Popielski (3) in a series of papers (1909–1913) questioned the specificity of pyloric mucosa extract, stating that the stimulating action was due to the more fluid nature of the blood and to vasodilatation and could be obtained from tissue extracts in general. Ehrmann (4) reported that acid extracts of pyloric, fundic and duodenal mucosa caused a flow of gastric juice when injected subcutaneously. Einsmann (5) reported stimulation of gastric secretion to follow the injection of extracts of pyloric mucosa, duodenal mucosa, liver jejunum, ileum and pancreas. Tomaszewski (6) found a gastric secretogogue to be present in the pyloric and fundic mucosa. Keeton and Koch (7) found gastrin to be distributed throughout the gastric mucosa, in the duodenum, the esophagus and brain. Luckhardt, Keeton and Koch (8) found secretogogue action in extracts of the duodenum, liver, pancreas, thyroid and Armour's scale pepsin. Rogers, Fawcett et al (9) reported marked secretogogue action in extracts of thyroid, liver, parathyroids, spleen, pancreas, thymus and pineal gland. Luckhardt, Keeton and Koch (8), however, found the extracts of the spleen, thymus, muscle, gastric juice and fibrin to be inactive. Ivy and Oyama (16) found that gastric mucus did not contain a secretogogue. Luckhardt, Keeton and

[1] Preliminary report: Proc. Amer. Physiol. Soc., Chicago, 1920.

Digitized by Google

Koch (10) also found that histamine when injected subcutaneously will stimulate gastric secretion and which in many respects is very similar to the stimulation produced by gastrin.

So the bulk of the evidence at hand shows that the gastric secretogogue in the extract of pyloric mucosa is not specific for the pyloric and cardiac mucous membranes but has a wide distribution, being present "in relatively high concentrations," according to Koch, Luckhardt and Keeton (10) "in the same organs in which ammonia is generally most prominent immediately after death."

With the exception of the work of Gross (2) in 1906 and of Edkins and Tweedy (11) in 1909, all of the investigations of this problem have been of a pharmacological nature, *i.e.*, extracts have been prepared and injected parenterally and their effects on gastric secretion noted.

It is generally recognized that as a rule it is not correct to assume that pharmacological action denotes physiological significance. Because an extract of the pyloric mucous membrane when injected parenterally stimulates gastric secretion, it does not follow that the active principle of the extract is physiologically important or significant.

In accordance with this principle Edkins and Tweedy (11) devised a method which enabled them to functionally separate the pyloric from the fundic end of the stomach and reported that when "different substances (HCl, dextrin, dextrose, meat extracts, peptone solution) were placed in the pyloric region of the stomach or in the duodenum in all cases the fundus responded by marked secretion." These investigators divided the stomach into two chambers by means of a partition which consisted of a rubber balloon, shaped like a pulley wheel, which was inserted through the pyloric orifice from the duodenum. The balloon when in position in the stomach was inflated and tied in place by passing a ligature about the stomach outside the muscle wall but beneath the blood vessels, just opposite the groove in the pulley-shaped balloon, thus dividing the stomach into two water-tight compartments. Thirty cubic centimeters of normal saline solution at 37°C. were then introduced into the fundic chamber, after it had been washed out, and the different substances were then introduced into the pyloric chamber at a pressure of 10 cm. water pressure. This was allowed to remain for 1 to 2 hours when the 30 cc. of normal saline solution were removed from the fundic compartment and titrated for degree of acidity. They report acid values varying from 0.0058 per cent to 0.16 per cent. Cats were used. At no place in their paper do they state that controls were made, that is, experiments in which normal saline solution was placed in the fundic

Digitized by Google

compartment and nothing in the pyloric compartment and 1 to 2 hours later titrating the normal saline solution for acidity. In other words, they failed to take into consideration the possibility of a continuous secretion of gastric juice. The only reason that we are able to assign for omitting controls is a statement Edkins makes in a previous article (1) to the effect that he was "able to show that normal saline solution introduced into the stomach would remain for a prolonged period (1 to 2 hours) unabsorbed and without change in reaction." This statement was based on some work he did in 1892 on cats under chloroform anesthesia and a hypodermic injection of $\frac{1}{2}$ grain of morphine and $\frac{1}{18}$ grain of atropine. And even when the cats were under this atropinized condition his protocols report that two out of five cats experimented upon (cats XI and XII) showed an acid reaction of the normal saline solution that had remained in their stomachs from 1 to 2 hours. The two cats in which the acid reaction occurred had food in their stomachs previous to washing it out, which is a factor of importance, as will be pointed out in our experimental work. Further, Edkins states (1) that "atropine does not diminish the reaction of an animal to this excitant" (gastric secretin) which has been shown to be wrong by Keeton, Luckhardt and Koch (12) in Pavlov pouch dogs and by Maydell (17), and these latter results have been confirmed in the course of our work. Hence this phase of the work of Edkins can hardly be considered conclusive.

Sokolov (13), working in Pavlov's laboratory on animals with a Pavlov pouch, an "obstructed" stomach, a duodenal and gastric fistula with a rubber tube connecting the two fistulae reported that food substances when injected into the duodenum of such an animal would not exert any influence on the gastric glands, but when injected into the "obstructed" stomach and retained there, secretion of the Pavlov pouch would occur. These results were interpreted as demonstrating that the secretion was excited by reflex effect from the gastric mucous membrane and that the chemical secretion of gastric juice is caused by an effect proceeding mainly from the inner surface of the stomach and not from the intestine (18).

Gross (2) also working in Pavlov's laboratory on an animal preparation similar to that of Sokolov, with the exception that the obstruction was made at the junction of the fundic mucosa and pyloric mucosa, obtained results that partially contradicted the findings of Sokolov and drew conclusions from his results that supported the "gastrin theory" of Edkins. The results of Gross show when meat extract is injected into the fundic portion of the stomach and retained there, no stimulation

Digitized by Google

of secretion results in the Pavlov pouch, but when the meat extract is injected into the duodenum through the duodenal fistula that a stimulation of the gastric glands results. Gross explains this contradiction of Sokolov's findings by assuming that the meat extract he (Gross) injected into the duodenum flows back into the pyloric antrum and there produces its effect. This assumption we do not believe to be plausible because in our experience the pyloric antrum readily and rapidly expels its contents, as we shall point out later in our experimental work. A second assumption is also necessary and that is that the elaboration of "gastrin" actually does occur when meat extract is in contact with the pyloric mucosa, which has not yet been proved.

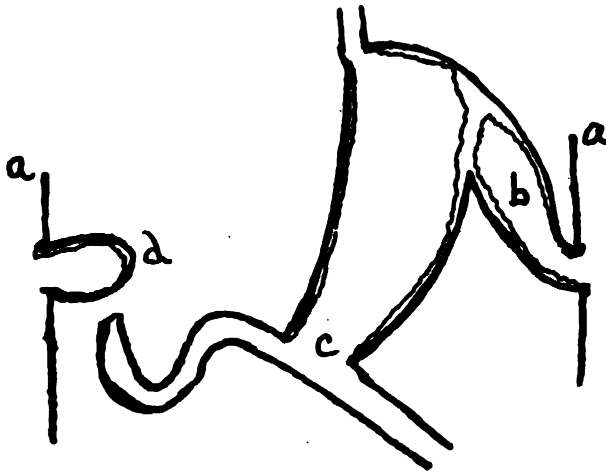


Fig. 1. *a*, abdominal wall; *b*, Pavlov pouch; *c*, gastro-duodenostomy; *d*, pyloric pouch.

Experimental: In the course of studies on the physiology of the pyloric secretion, animals with two gastric pouches (14), one a Pavlov pouch and the other a pouch of the entire pyloric antrum, were prepared (see fig. 1) so that the secretions from the fundic and pyloric mucous membranes could be collected simultaneously. It occurred to us that such an animal might be used to demonstrate the reality of the "gastrin theory," in which we were firm believers, because it was possible to apply substances to the pyloric mucosa in healthy or physiological animals and to observe changes in the secretion of the Pavlov pouch.

Method: Our method consisted in collecting two to three hours of continuous secretion (24 hours after the last meal) from the Pavlov pouch and then in applying the various substances for a period of 2

Digitized by Google

hours to the mucosa of the pyloric pouch and at the same time continuing the collection of secretion from the Pavlov pouch. Application of the substances was made by injecting the substances into the pouch and then plugging the orifice with cotton, this being repeated every 3 to 5 minutes. In order to insure continuous contact, as the pouch would contract and expel its contents, another device was made which alleviated this trouble.

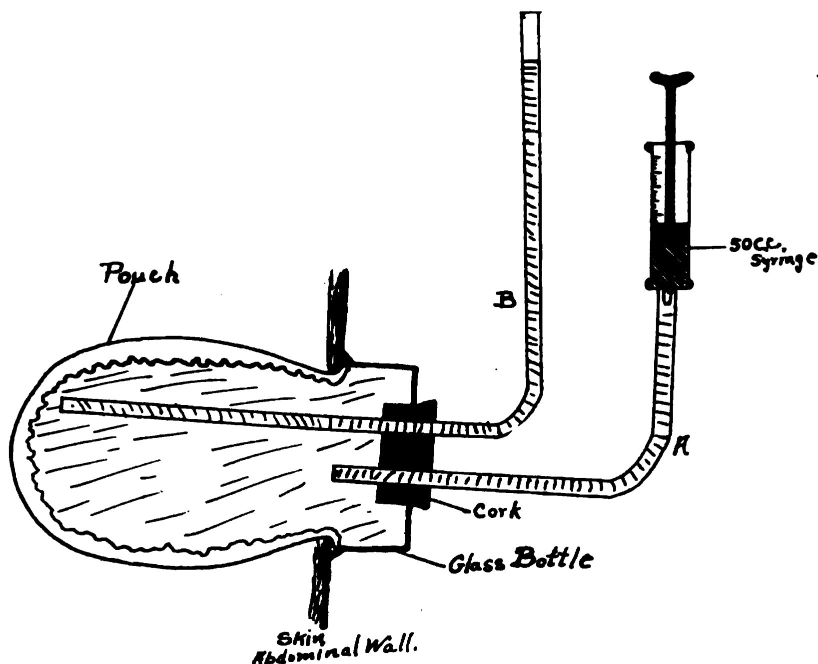


Fig. 2

The device consists of a glass bottle, a cork and a glass tube arranged as in figure 2. The solution to be applied is put into a 50 cc. syringe and injected slowly through tube *A*, so that all the air will be displaced from the bottle and the pouch through tube *B*, until the solution stands in tube *B* above the level of the pouch. The level of the solution in tube *B* and in the syringe varies with each respiration and is raised markedly with each contraction of the pouch. When the musculature of the pouch relaxes again the displaced fluid passes back into the pouch.

Four out of six dogs subjected to our operation lived in good health for 1 month or longer. The first dog operated only lived 16 days and

Digitized by Google

only a few tests were made on him. The second dog lived 3 weeks, the third dog 2 months, the fourth dog 5 weeks, the fifth dog 4 months and the sixth dog 7 months. Dog VI died 1 month after making a duodenal fistula. All of the dogs died of a similar trouble, the cause of which we have no definite explanation, the chief symptoms being loss of weight and anorexia, beginning 10 to 15 days prior to death—all dogs dying of intercurrent pneumonia. Anemia, associated with bleeding of the gums, was present in all of the animals prior to death. At autopsy no lesions were found other than broncho-pneumonia, which was assigned as the immediate cause of death.

Results: All of the dogs at the time the various substances were applied to the pyloric mucosa reacted normally to a standard meal of meat and to a deep injection of gastrin. In dog I only three tests were made. In all the other dogs many tests were performed using $\frac{N}{10}$ HCl, gastric juice, gastrin, fresh meat extract and Liebig's meat extract. Dextrose and peptone solutions were used twice in one animal with negative results.

The results were negative for all of the substances used, table 1 demonstrating the absence of response when fresh meat extract was applied to the mucosa of the pyloric pouch and the routine of procedure followed in all of our experiments. Further tables of results are omitted because of their negative nature. We desire to emphasize that after the application of the substance under test to the mucosa of the pyloric pouch the animal was given or injected with gastrin in order to demonstrate that the gastric secretory mechanism of the animal at the time of application was normal. We also desire to call attention to the necessity of following the continuous secretion of the Pavlov pouch before and after a certain procedure in order to determine whether or not the procedure caused stimulation.

Repetition of Edkins and Tweedy's experiments. Having failed to demonstrate the reality of the "gastrin theory" with our "two gastric pouch" preparation, we sought an explanation. We were convinced that Edkins and Tweedy (11) did not adequately control their experiments as has been pointed out in the first part of our paper. The work of these investigators was then repeated.

Method: We at first attempted to use the technique devised by Edkins and Tweedy, but in inserting the balloon more trauma was produced than we thought would be good for the results of the experiment. So we used a more simple technique—one that is frequently used—which consisted in tying a ligature about the cardiac orifice, the pyloric sphinc-

Digitized by Google

ter and a third one at the transition between fundic and pyloric mucous membrane. The ligatures were passed beneath the blood vessels. Our only variation from the technique of the above investigators then was to dispense with the use of the balloon. All solutions were warmed to 37°C. before using and the stomach was washed out before the introduction of the normal saline solution. Light ether anesthesia was used.

Results: The results (table 2) demonstrate that there is an increased acidity of the normal saline solution that is put in the fundic chamber within the same limits as occurs when 15 cc. of meat juice or Liebig's

TABLE 1

*Fresh meat extract solution applied to mucosa of pyloric pouch *dog III*

PROCEDURE	TIME	PAVLOV POUCH SECRETION	TOTAL ACIDITY	FREE ACIDITY	REMARKS
	o'clock	*cc.*	*per cent*	*per cent*	
Continuous secretion	10–11	1.0	0.2188	0.0912	
Continuous secretion	11–12	1.7	0.3098	0.2553	
Continuous secretion	12–1	2.0	0.3098	0.2553	
Fresh meat extract solution applied to pyloric pouch	1–2	2.2	0.2553	0.2188	Extract of 1 pound of lean beefsteak
Application continued	2–3	2.1	0.1459	0.1094	
Continuous secretion	3–4	2.0	0.1641	0.0730	
Test meal					
First hour	4–5	4.1	0.2918	0.1842	½ pound of lean beef and 200 cc. water
Second hour	5–6	10.0	0.3737	0.2918	
Third hour	6–7	5.0	0.4193	0.3463	

* The fresh meat extract was made by boiling 1 pound of ground lean beefsteak for 5 minutes in 200 cc. of water and then expressing the juice. The mixture was raised to the boiling point slowly in from 20 to 30 minutes.

meat extract are put in the pyloric chamber. The slight increase in acidity that occurs in both cases we believe to be due to the continuous secretion of the stomach. It is to be recalled that the highest acidity reported by Edkins and Tweedy is 0.16 per cent which we believe to be within the normal, even though our highest per cent of acidity is 0.13 per cent. The results on the dog are the same as those on the cat with the interesting difference that the secretory mechanism in the cat is more resistant to ether anesthesia and the experimental procedure than in the dog.

Digitized by Google

ABSORPTION FROM THE MUCOSA OF THE PYLORIC POUCH. The question of absorption from the mucosa of the pyloric pouch must be considered as it is possible that absorption may not occur at as fast a rate

TABLE 2

PROCEDURE	ANIMAL NUMBER	TOTAL ACIDITY OF N. S. S. TAKEN FROM THE STOMACH	AVERAGE TOTAL ACIDITY OF N. S. S. TAKEN FROM STOMACH
		per cent	*per cent*
Six dogs: Control, 50 cc. N.S.S. placed in fundic chamber	1	0.018	
	2	0.036	
	3	0.082	
	4	0.027	0.046
	5	0.054	
	6	0.036	
Six dogs: 50 cc. N.S.S. placed in fundic chamber and 15 cc. of meat extract in pyloric chamber	11*	0.073	
	12	0.054	
	13	0.018	0.042
	14	0.027	
	15	0.045	
	16	0 036	
Eight cats: Controls 30 cc. N.S.S. placed in fundic chamber	1*	0.091	
	2	0.018	
	3	0.063	
	4*	0.136	0.072
	5	0.054	
	6	0.082	
	7	0.136	
	8	0.045	
Six cats: 30 cc. N.S.S. placed in fundic chamber and 10–15 cc. of meat extract in pyloric chamber	11	0.027	
	12	0.054	
	13	0.136	0.070
	14*	0.127	
	15*	0.127	
	16*	0.091	

* The stomach was full of food. In all cases the stomach was washed out with from 300 to 500 cc. of N.S.S. at 37°C.

as it does in the pyloric mucosa when it is in its normal anatomical position. It is possible that the procedure of making the pouch might have in some way altered absorption from the mucosa of the pouch.

Digitized by Google

This question cannot be answered directly for any of the solutions used in our experiments, because with present methods it is practically impossible to prove without question whether or not absorption of the substances applied occurred, with the possible exception of dextrose and acid. We have attempted to answer the question indirectly by comparing the rates of absorption of strychnine sulphate, pilocarpine hydrochloride and potassium iodide from the mucosa of the pyloric pouch and from the mucosa of the pyloric antrum in situ.

Results: Solutions of strychnine sulphate (3 per cent), pilocarpine hydrochloride (100 per cent) and potassium iodide (100 per cent) were used. They were applied to the mucosa of the pouch in the usual manner. In the case of the strychnine, the time of occurrence of increased reflexes and of spasms was observed. In the case of pilocarpine hydrochloride, the time of salivation was noted. When potassium iodide was applied, the time of its appearance in the saliva and gastric juice was noted,[2] the starch test being used. The solutions were applied to the mucosa of the pyloric antrum in its normal position by anesthetizing the dog, ligating off the pyloric antrum with two ligatures, which were placed beneath the blood vessels, and by injecting the solution into the lumen of the pyloric antrum by means of a syringe, after which the abdomen was closed. Care must be exercised to have the needle of the syringe clean and not to spill any of the solution on the serosa of the stomach. In the case of strychnine (10 cc. of a 3 per cent solution) the ether was discontinued and the time of occurrence of increased reflexes and of spasm was observed. In the case of pilocarpine (1 cc. of a 100 per cent solution, Wharton's duct was cannulated and the time of the beginning of salivation noted. In the case of potassium iodide (5 cc. of a 100 per cent), Wharton's duct was cannulated and the chorda-tympani nerve stimulated every 10 minutes and the time of the appearance of potassium iodide in the saliva was noted.

When strychnine is applied to the pouch, the reflexes are definitely increased in from 15 to 20 minutes and spasms appeared in from 20 to 25 minutes. When styrchnine is injected into the pyloric antrum in situ, increased reflexes occur in from 20 to 35 minutes and spasms appear in from 35 to 45 minutes. Pilocarpine when applied to the mucosa of the pyloric pouch, causes salivation in from 3 to 5 minutes and vomiting and defecation in from 5 to 7 minutes; when injected into the pyloric antrum in situ, it causes salivation in from 10 to 15 minutes. Potassium iodide, when applied to the mucosa of the pyloric pouch, makes

[2] "Two gastric pouch" dogs used.

Digitized by Google

its appearance in the gastric juice in from 15 to 30 minutes and in the saliva, provided the dog's salivation is stimulated by pilocarpine injection, in from 1½ to 3 hours. When potassium iodide is injected into the pyloric antrum in situ, it appears in the saliva in from 1 to 2 hours and in the "fundic" secretion in from 15 to 30 minutes.

These data demonstrate that the rate of absorption of the mucosa of the pyloric pouch has not been altered by the operative procedure and that the absorption of potassium iodide and of strychnine from the pyloric mucosa is comparatively slow.

SUMMARY

We made the "two gastric pouch" preparation in the hope of demonstrating the "gastrin theory." This theory maintains that food substances in contact with the mucosa of the pyloric antrum cause the formation of a hormone, gastrin, which is absorbed into the blood stream and carried to the glands of the "fundic" mucosa and stimulates them. Our results do not support the gastrin theory. We failed to obtain an increase in the secretion of the Pavlov pouch when various substances, including food extracts, were applied to the mucosa of the pyloric pouch, which should have occurred according to the "gastrin theory."

Not being able to find fault with our preparation—the animals having been proven to be physiological in every respect—and experiments, we repeated the work of Edkins and Tweedy and have demonstrated that they did not adequately control their experiments. This deficiency of proper controls has also been pointed out by other investigators (3), (4), (5), (6), (7), (8), (9) and confirmed by us with reference especially to the claims of the "specificity of gastrin."

BIBLIOGRAPHY

(1) Edkins: Journ. Physiol., 1906, xxxiv, 133.
(2) Gross: Arch. Verdaungskrankheiten, 1906, xii, 507.
(3) Popielski: Pflüger's Arch., 1909, cxxvi, 483; Ibid., 1913, cl, 1.
(4) Ehrmann: Inter. Beitr. z. Path. u. Therap. d. Ernährungsstörungen, 1912, iii, 382.
(5) Emsmann: Inter. Betr. z. Path. u. Therap. d. Ernährungsstörungen, 1912, iii, 117.
(6) Tomaszewski: Zentralbl. Physiol., 1913, xxvii.
(7) Keeton and Koch: This Journal, 1915, xxxvii, 481.
(8) Luckhardt, Keeton and Koch: This Journal, 1920, l, 527.
(9) Rogers, Fawcett et al: This Journal, 1915, xxxvii, 453; 1915, xxxix, 345, 154; 1919, xlvii, 79.

Digitized by Google

(10) Koch, Luckhardt and Keeton: This Journal, 1920, lii, 508.
(11) Edkins and Tweedy: Journ. Physiol., 1909, xxxviii, 263.
(12) Keeton, Luckhardt and Koch: This Journal, 1920, li, 469.
(13) Sokolov: Inaug. Dissert. St. Petersburg, 1904. The work of the digestive glands, London, 1910, 114.
(14) Ivy: Arch. Int. Med., 1920, xxv, 6.
(15) Ivy: Journ. Amer. Med. Assoc., 1920, lxxv, 1540.
(16) Ivy and Oyama: This Journal, 1921, lvii, 51.
(17) Maydell: Dissertation, Kiev, 197. Physiol. Abst., 1917-18, ii, 146.
(18) Pavlov: The work of the digestive glands, 1910, London.

Digitized by Google

INDEX TO VOLUME LX

ADRENALS, relation of, to fatigue, 255.
Albino mice, adaptation of, to tropical environment, 397, 416, 425, 434, 443.
Alcohols, polyhydric, narcotic effect of, 496.
——, stimulant action of, 151, 270.
——, successive stimulation by, 274.
Alkaligenesis in tissues, 519.
AMBERG, S. and M. E. MAYER. The isolation of a substance from urine having properties of citric acid. Description of an apparatus facilitating the working with small volumes of gas, 564.
Amoebocyte tissue, factors influencing outgrowth of, 277.
Anemia, cerebral, cardio-vascular changes during, 1.
Anoxemia, extreme, cardiac changes during, 155.
ASCHAM, L. See SMITH and ASCHAM, 250.
Asphyxia, carbon monoxide, 244.
Autonomic fibers, regeneration of, 350.

BAKWIN, H. The significance of hydremia in the secretion of urine, 343.
BASSETT, S. H., E. HOLT and F. O. SANTOS. The influence of meat upon physical efficiency, 574.
BERCOVITZ, Z. Studies on the visceral sensory nervous system. XII. The response of the isolated esophagus of the frog and the turtle to certain drugs, 219.
Beriberi, water content of tissues in, 234.
Bile salts and pigments, variations in output of, 119.
BLANCHARD, K. C. See LOEB and BLANCHARD, 277.
Blood morphology of mice, effect of humid heat on, 443.
—— pressure response to tissue compression, 323.
—— —— waves produced by slow and rapid breathing, 500.
—— serum, influence of, on coagulative activity of tissue extracts, 193.
Body form and surface area, relations of, to tropical environment, 416.
BURGET, G. E. and M. E. STEINBERG. Studies on the physiology of gastro-enterostomy. I. The regurgitation of intestinal contents in normal dogs and dogs with posterior gastro-enterostomy, 308.
BURLAGE, S. R. A study of the regeneration of the autonomic fibers in the vagus nerve of the sheep, 350.

CANNON, W. B. and P. E. SMITH. Studies on the conditions of activity in endocrine glands. IX. Further evidence of nervous control of thyroid secretion, 476.
—— and F. R. GRIFFITH. Studies on the conditions of activity of the endocrine glands. X. The cardio-accelerator substance produced by hepatic stimulation, 544.
Carbon monoxide asphyxia, 244.
Cardiac changes during extreme anoxemia, 155.
Cardio-accelerator substance produced by hepatic stimulation, 544.
Cardio-respiratory interference waves, 500.
Cardio-vascular changes during cerebral anemia, 1.

Digitized by Google

Circulation, response of, to low oxygen tension, 155.
Citric acid, substance in urine with properties of, 564.
Climate, tropical, effect of, 397, 416, 425, 434, 443.
Coagulative action of tissue extracts, influence of blood serum on, 193.
CRILE, G. W., H. R. HOSMER and A. F. ROWLAND. The electrical conductivity of animal tissues under normal and pathological conditions, 59.
CUNNINGHAM, R. S. Studies in placental permeability. II. Localization of certain physiological activities in the chorionic ectoderm in the cat, 448.

DAVIS, L. E. Vasomotor responses obtained by slowly interrupted faradic stimulation of the thoracic sympathetic nerve, 560.
DEGENER, L. M. Studies on the effect of diet on the weight of the hypophysis and thyroid gland of the albino rat, and on the action of their extracts on the isolated small intestine, 107.
Diet, effect of, on hypophysis and thyroid gland, 107.
Dietary, significance of meat in, 574.
Duodenal contraction by sodium carbonate, 48.

EDSON, P. See TROTTER, EDSON and GESELL, 500.
Electrical conductivity of animal tissues, 59.
Endocrine glands, conditions of activity in, 476, 544.
Epinephrin, liberation of, in traumatic shock, 461.
Esophagus, isolated, response of, to certain drugs, 219.

FATIGUE, relation of adrenals to, 255.
FEIL, H. and D. D. FORWARD. The pulse in the portal vein, 312.
FORWARD, D. D. See FEIL and FORWARD, 312.

GASTRIN theory, physiological test of, 578.
Gastro-enterostomy, physiology of, 308.
GESELL, R. See TROTTER, EDSON and GESELL, 500.
GILBERT, N. C. See GREENE and GILBERT, 155.
GREENE, C. W. and N. C. GILBERT. Studies on the response of the circulation to low oxygen tension. VI. The cause of the changes observed in the heart during extreme anoxemia, 155.
GRIFFITH, F. R. See CANNON and GRIFFITH, 544.
Growth and fertility of mice, effect of tropical environment on, 397.
—— —— pigmentation of hair, effect of tropical climate on, 425.
——, appetite and, after splenectomy, 250.
—— of neuroblast in presence of carbon monoxide, 244.
GULICK, A. A study of weight regulation in the adult human body during over-nutrition, 371.

H- and OH-ions in intestinal activities, 52.
HAGGARD, H. W. Studies in carbon monoxide asphyxia. II. The growth of neuroblast in the presence of carbon monoxide, 244.
HAMMETT, F. S. The rôle of the change in hydrogen-ion concentration in the motor activities of the small intestine, 52.
—— and J. E. NOWREY, JR. The rôle of the sodium and the carbonate ions and of the change in the sodium-calcium ratio in the concentration of the isolated duodenal segment of the albino rat, 48.

Digitized by Google

HARTMAN, F. A., R. H. WAITE and E. F. POWELL. The relation of the adrenals to fatigue, 255.
HASHIMOTO, H. The secretory action of the pancreas in relation to the thyroid gland:
I. The effect of thyroid feeding in rats upon the secretory action of the pancreas, 357.
II. The effect of thyroidectomy in rats upon the secretory action of the pancreas, 365.
Hepatic stimulation, cardio-accelerator substance produced by, 544.
HOLT, E. See BASSETT, HOLT and SANTOS, 574.
Hormone, nervous and, control of respiratory center, 134.
HOSMER, H. R. See CRILE, HOSMER and ROWLAND, 59.
Hydremia in secretion of urine, 343.
Hypophysis, effect of diet on, 107.

INTESTINAL activities, H- and OH- ions in, 52.
—— contents, regurgitation of, after posterior gastro-enterostomy, 308.
IRWIN, M. Sensory stimulation by saturated monohydric alcohols, 151.
——. Sensory stimulation by unsaturated alcohols, polyhydric alcohols and chlorhydrins, 270.
——. Successive stimulation by alcohols, 274.
IVY, A. C. and J. E. WHITLOW. The gastrin theory put to physiological test, 578.

KLEITMAN, N. Studies on the visceral sensory nervous system. XI. The action of cocaine and aconitine on the pulmonary vagus in the frog and in the turtle, 203.
KRAUSE, D. J. The water content of the tissues in experimental beriberi, 234.

LIGHT and heat, effect of, on resistance of mice to acetonitrile, 434.
LOEB, L. and K. C. BLANCHARD. The effect of various salts on the outgrowth from experimental amoebocyte tissue near the isoelectric point and with the addition of acid or alkali, 277.

MACHT, D. I. and G. C. TING. The effect of some polyhydric alcohols on the behavior of rats in the circular maze, 496.
MACLEOD, J. J. R. and S. U. PAGE. The relationship between nervous and hormone control of the respiratory center, 134.
MARTIN, K. A. and H. L. WHITE. Blood pressure responses to hypersystolic compression of tissues, 323.
MATHEWS, S. See MILLS and MATHEWS, 193.
MAYER, M. E. See AMBERG and MAYER, 564.
MILLS, C. A. and S. MATHEWS. Influence of blood serum on the coagulative activity of tissue extracts, 193.

NERVE fiber during excitation, ammonia production in, 519.
——, thoracic sympathetic, vasomotor responses from, 560.
Nervous and hormone control of respiratory center, 134.
—— control of thyroid secretion, 476.
—— system, visceral sensory, 203, 219.
—— tissue, action of carbon monoxide on, 244.
NOWREY, JR., F. E. See HAMMETT and NOWREY, 48.

OVER-NUTRITION, weight regulation in man during, 371.
Oxygen tension, low, responses of circulation to, 155.

PAGE, S. U. See MACLEOD and PAGE, 134.

Digitized by Google

Pancreatic function, relation of thyroid to, 357, 365.
Physical efficiency, influence of meat on, 574.
Pigments, bile salts and, variations in output of, 119.
Placental permeability, 448.
Portal vein, pulse in, 312.
POWELL, E. F. See HARTMAN, WAITE and POWELL, 255.
Pulse in the portal vein, 312.

RAPPORT, D. Studiesin experimental traumatic shock. VI. The liberation of epinephrin in traumatic shock, 461.
Respiratory center, nervous and hormone control of, 134.
ROWLAND, A. F. See CRILE, HOSMER and ROWLAND, 59.

SANTOS, F. O. See BASSETT, HOLT and SANTOS, 574.
Sensory stimulation by saturated monohydric alcohols, 151.
Shock, experimental traumatic, 461.
SMITH, A. H. and L. ASCHAM. The relation of splenectomy to growth and appetite in the rat, 250.
SMITH, P. E. See CANNON and SMITH, 476.
Splenectomy, appetite and growth after, 250.
STEINBERG, M. E. See BURGET and STEINBERG, 308.
SUNDSTROEM, E. S. Studies on the adaptation of albino mice to an artificially produced tropical climate:
I. Effect of the various factors composing a tropical climate on growth and fertility of mice, 397.
II. Relations of the body form and especially the surface area to the reactions released by and the resistance to a tropical climate, 416.
III. Effect of the tropical climate on growth and pigmentation of hair and the dependence of these integumental functions on the temperature coefficient law, 425.
IV. Effect of light and heat on the resistance of mice to acetonitrile, 434.
V. Effect of humid heat on the blood morphology of mice, 443.

TASHIRO, S. Studies on alkaligenesis in tissues. I. Ammonia production in the nerve fiber during excitation, 519.
Thyroid gland, effect of diet on, 107.
——, relation of, to pancreatic function 357, 365.
—— secretion, nervous control of, 476.
TING, G. C. See MACHT and TING, 496.
Tissue, amoebocyte, factors influencing outgrowth of, 277.
—— compression, blood pressure response to, 323.
—— extracts, influence of blood serum on coagulative activity of, 193.
Tissues, alkaligenesis in, 519.
——, animal, electrical conductivity of, 59.
——, water content of, in beriberi, 234.
TROTTER, R. T., P. EDSON and R. GESELL. A comparison of waves of blood pressure produced by slow and rapid breathing, 500.

URINE, hydremia in secretion of, 343.
——, substance in, with properties of citric acid, 564.

VAGUS, pulmonary, action of cocaine and aconitine on, 203.
—— regeneration, 350.
Vasomotor responses from thoracic sympathetic nerve, 560.
Visceral sensory nervous system, 203, 219.

Digitized by Google

WAITE, R. H. See HARTMAN, WAITE and POWELL, 255.

Water content of tissues in beriberi, 234.

Weight regulation in man during over-nutrition, 371.

WHIPPLE, G. H. See WISNER and WHIPPLE, 119.

WHITE, H. L. See MARTIN and WHITE, 323.

WHITLOW, J. E. See IVY and WHITLOW, 578.

WINKIN, C. S. An analysis of the nervous control of the cardiovascular changes during occlusion of the head arteries in cats, 1.

WISNER, F. P. and G. H. WHIPPLE. Variations in output of bile salts and pigments during 24-hour periods, 119.

Digitized by Google

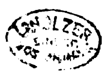
Digitized by Google

Digitized by Google

Digitized by Google

Digitized by Google

Digitized by Google



Digitized by Google

Digitized by Google